A DETAILED STUDY OF THE CHANGES OCCURRING IN THE PHYSIOLOGICAL DEGENERATION OF ACTINOSPHÆRIUM EICHORNI.*

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INTRODUCTION.

For a clear understanding of the changes to be described in the series of *Actinosphæria,* the study of which forms the basis of this communication, it is necessary to give a description of a normal *Actinosphærium* and a resumé of Richard Hertwig's work on the physiological degeneration of this animal.

In life, a normal *Actinosphærium* is a spherical cell with numerous radially projecting pseudopodia, regularly distributed over the surface, and each with a homogeneous axis fiber. The protoplasm is sharply divided into a coarsely vacuolated cortical layer, which contains the contractile vacuoles and through which the bases of the axis fibers of the pseudopodia run; and an inner, more finely vacuolated protoplasm, the medulla, in which lie the nuclei and the food vacuoles. The nuclei are situated chiefly in the peripheral layer of the medulla, just beneath the cortex; but a few nuclei are scattered in the deeper parts of the medulla. The number of nuclei varies with the size of the animal. According to R. Hertwig, the nuclei, under normal conditions, vary from 10 to 14 μ in diameter, and are surrounded by a distinct nuclear membrane, and contain nuclear sap and an achromatic linin framework, on which lie the nucleolar substance and the chromatin. He regards the nucleolar substance as identical with the material of which the nucleoli of the tissue cells of metazoa consists. According to Hertwig, in the simplest, but least common conditions, as

* Received for publication November 25, 1907.

in functional rest and just before division, the nucleolar substance is mixed with the chromatin into a spherical nucleolus or amphinucleolus. Usually, the material of the amphinucleolus is divided, either into two or more small nucleoli, or into dendritically branching processes, which radiate from the center to the periphery and lie on the nuclear framework. The latter arrangement is an indication of energetic nuclear function. He describes nuclei with divided amphinucleolus, in which there is a separation of nucleolar substance and chromatin. In these, the chromatin seeks the peripheral ends of the processes, so that the center of the figure, which he calls the chromatin rosette, appears light.

In his work on the physiological degeneration of *Actinosphærium eichorni,* Richard Hertwig ('04) described a series of important changes affecting the structures of this rhizopod which shed much light upon both the physiology and the pathology of the cell. These may be summarized as follows:

(*a*) *Chromidium.*—In 1902, he first noticed the presence in the plasma of *Actinosphærium* of fine or coarse granules, rods, or even triangular and slightly branched bodies, with the staining reactions of the amphinucleolus and consisting of a mixture of chromatin and nucleolar substance. These masses, the origin of which Hertwig traced to the nucleus, lie both on the plasmatic meshwork and in the vacuoles of the same, and were called chromidia. They occur chiefly in the medulla, but may be present in the cortex. They may be present in starved, as well as in overfed, *Actinosphæria.* Hertwig draws an important distinction between the chromidia above mentioned and the "Chromidialnetz" which he had previously described in the Thalomorphora; the latter he regards as the chief seat of the functional capacity of the cell, and the former he considers superabundant functionless parts of the nucleus which are thrown off to be got rid of, just as the chromidia of egg cells. It is necessary to emphasize here the distinction between chromidia in this sense and the functionally active forms of extranuclear chromatin known variously as "Chromidia," "Chromidialnetz" and "Chromidialapparatus," which, as R. Hertwig, Schaudinn and Goldschmidt have shown, play an important rôle in many protozoan and metazoan cells.

(*b*) *Pigment Formation.*—This occurs in *Actinosphærium* under various conditions in which a considerable amount of nuclear material is destroyed, as just before encysting and in starved cultures. The highest degree of pigmentation occurs in the depression of overfeeding, in connection with marked production of chromidia. The pigment, which Hertwig has traced to the destruction of chromatin, is of a yellowish brown color, and consists of fine refractile particles, which may be isolated or in smaller or larger clumps in the medulla and cortex. The pigment granules often lie about the nuclei. They often beset the peripheral layer of the cortex, from which they are extruded. They may also lie in sequestra, which may be thrown out.

(*c*) *Destruction of Nuclei.*—Of this Hertwig describes three types. (1) Simultaneous solution of all the nuclei to chromidia. (2) The transformation of one or a few nuclei to giant nuclei, which may be thrown out, while the rest of the nuclei are dissolved. (3) The extrusion of the whole medulla with its nuclei.

1. The transformation of all the nuclei to chromidia with the disappearance of the other nuclear constituents was observed first in three animals in an encysting culture; in the place of nuclei they were beset with chromidia. Hertwig explained the phenomenon as due to excessive development on the part of the protoplasm of its normal capacity to destroy nuclei when encysting. Under normal conditions, in encysting *Actinosphæria,* only from ninety to ninety-five per cent. of all the nuclei are dissolved, while in these animals all the nuclei were destroyed.

In his overfeeding experiments also, he produced "chromidial animals" with solution of nuclei. In this instance, he explained the changes on the basis of his well known "Kern-plasma-relation theory."

2. The formation of giant nuclei occurs during the depression associated with overfeeding. The animals are the seat of other changes, such as the loss of pseudopodia and of the distinction between cortex and medulla. The giant nuclei, which are discernible in the living animal, may reach the diameter of 196 μ. The giant nuclei are surrounded by a radiating zone of fine granules. An animal may develop from fifteen to twenty giant nuclei.

Giant nuclear animals die in a few days, but before death one or more of these nuclei may be thrown off. In stained preparations, giant nuclei show a separation of the chromatin containing nuclear mass and the chromatin free portions; the latter form one or two round fluid-containing vesicular bodies which lie in the chromatin rosette.

Then the nucleoli increase in size, become vesicular, and take on a reticulated appearance. With the growth of the nucleolar bodies, the nuclear reticulum is pushed to the periphery and disappears. The remains of the chromatin rosette are condensed; if there are two nucleolar bodies, the chromatin lies between them, if only one, the chromatin mass is compressed at one side, giving the appearance of a signet-ring. Rarely, the chromatin rosette is absorbed during the growth of the nucleus, leaving the nucleolar body without a chromatin rosette. The nuclei may increase in size from the usual diameter of 10 to 14 μ to 70 or even 196 μ, and thus in a few days the nuclear mass may be increased by from 125 to 3,000 times. The fusion of nuclei plays no part in the production of giant nuclei. The chief cause is a growth of the nucleolar substance. Deep depression does not always end in giant nucleus formation; it occurs most often after depressions at the termination of which the *Actinosphæria* have been able to multiply for weeks and months. Hertwig regards the giant nucleus formation in *Actinosphærium* as the result of a series of changes which lead to the growth of the nuclei at the cost of the protoplasm. The growth of nuclei has, then, increased for so long, that finally their growth intensity renders a normal course of life processes impossible, and death results. The formation of giant nuclei would be favored, therefore, by excessive cell function.

3. Hyperplasia and hypertrophy of nuclei occurred in a depressed culture of *Actinosphæria,* containing a few animals with giant nuclei; most of the animals were of small or of medium size, with numerous pseudopodia and well defined cortex and medulla. These animals showed an enormously increased number of abnormally small nuclei (from one-half to three-fourths the normal size). Otherwise they had the usual structure of *Actinosphærium* nuclei. This condition coincided with a period of excessive feeding and

active multiplication, and during a whole month (April) the animals were characterized by the great number and the smallness of their nuclei.

This period was followed by one in which the nuclei were increased in size, with their chromatin divided into branching strands. These "hypertrophied" nuclei differ from the giant nuclei in their smaller size and in the absence of such great changes in the nuclear structure.

Besides the "hypertrophied" nuclei these *Actinosphœria* contain recently divided nuclei and nuclei of more or less normal size and structure. The "hypertrophied" nuclei are often oval in form and, in diameter, measure in the whole animal, from 21 to 35 μ, rarely 40 μ.

In some of these nuclei the chromatin is spread out over the whole nuclear network—hyperchromatic nuclei. In others, numerous chromatic nucleoli with short radiating processes lie on the reticulum. In the third type there are numerous sharply circumscribed vacuolated chromatic nucleoli. Transition stages are distinguishable, from nuclei with a few chromatic vesicles, to nuclei with a single large vacuolated nucleolus.

The nuclear framework varies from a fine webbed, delicate, granular network to one with coarse, homogeneous meshes. The nuclear meshwork is always sharply separated from the surrounding plasma by a membranelike thickening which is a further distinction between the "hypertrophied" and the "giant" nucleus.

Hertwig also describes chromatin-free nuclei, which usually contain a central achromatic nucleolus. The chromatin of these nuclei has degenerated and the nuclear framework becomes gradually shrunken.

The protoplasm of *Actinosphœria* with hypertrophied nuclei may be covered, in the living animal, with fat drops. In fixed and stained animals, it is purple or brown, owing to the presence of very finely divided chromatin. Chromidia are absent. Not infrequently, the nuclei are in sausage-like strings. Finally, it often happens that parts of such animals may become necrotic and be cast off as brown multinuclear masses.

Hertwig brings forward evidence to show that these "hyper-

trophied" nuclei may divide and may also play a part in the functions of the animal; they may also become reduced to small nuclei.

In these *Actinosphæria,* depressed by overfeeding over long periods of time, in cultures under observation for months, R. Hertwig has described a remarkable series of changes in structure, comprising shrinking, fusion and disappearance of pseudopodia, loss of distinction between cortex and medulla, chromidia and pigment formation, the presence of fat, the occurrence of areas of necrosis, the extrusion of nuclei, the degeneration of nuclei, as well as various modifications in the size, number and structure of the nuclei.

In these cultures there were periods of great depression, followed by intervals of regeneration of more or less great degree, as shown by return, more or less complete, to normal structure. Hertwig lays stress upon the fact that these various phenomena are associated with changes of the nuclear apparatus, the basis of which is "an increase of the nuclear substance at the expense of the protoplasm," a condition which occurs in connection with periods of excessive overfeeding and which must be referred to the increased capacity to assimilate food. Thus, both hyperplasia and hypertrophy of nuclei results from increased functional activity. According to Hertwig's ('04) view, the nuclear mass of a cell increases in amount by taking substances from the protoplasm, which has the power of splitting up substances suitable for the formation of secretions and which are taken up into the nucleus.

As long as the movement of substances from the protoplasm to the nucleus is in the ascendency, the existence of the nucleus is assured; while a preponderance in the passage of materials from the nuclei leads to a partial or total dissolution of these structures. The nuclear hypertrophy may be brought about by overgrowth or fusion of nuclei; and the increase of nucleolar substances is the chief factor in the enlargement of the nuclei.

It is evident that in these degenerative changes described by R. Hertwig in *Actinosphæria* depressed by overfeeding, the equilibrium between nuclear mass and protoplasm is markedly disturbed and that there must exist a struggle for ascendency between the two forces which under normal conditions work in coöperation.

This leads to his " Kernplasmarelation " theory, according to which, " for each cell there exists a definite size-relation of nuclear mass to cell mass, which may be represented by the formula K/P (nuclear mass divided by protoplasm mass)" ('03). As the variations of the " Kernplasmarelation " depend, above all, upon the assimilation capacity of the cell and upon cell division, he distinguishes a functional and a division growth of the nucleus. It is the functional growth of the nuclear mass which concerns us here. His studies on protozoa have led R. Hertwig ('03) to the conclusion that the " Kernplasmarelation " underlies not only periodic modifications brought about by the ordinary processes of life, but that under certain influences, it may experience more lasting changes.

These influences are: (1) uninterrupted function, (2) hunger, (3) change of temperature.

The marked effects of these influences upon the " Kernplasmarelation " Hertwig and his pupils have demonstrated by experiments not only upon *Actinosphærium, Paramœcium, Dileptus gigas* and *Frontonia,* but upon the egg cells of various metazoa.

Hertwig's view of the influence of temperature upon the " Kernplasmarelation," has been confirmed by the interesting work of H. Marcus ('06) on the fertilized eggs of *Strongylocentrotus lividus.* Marcus developed the eggs at 9° C., 19° C. and 22° C., and found that, in the blastula stage, the nuclei of those bred in the cold were distinctly larger in proportion to the protoplasm than of those bred at the higher temperatures.

Returning to *Actinosphærium,* it has been shown that the " Kernplasmarelation " is distinctly disturbed in hunger, in overfeeding and in encysting; in hunger by decrease in the protoplasm, in overfeeding by the functional increase of the nuclear mass; and in encysting by an increase on the part of the protoplasm of its property of dissolving nuclei.

SPECIAL PART.

The *Actinosphæria* upon which the following observations are based were taken by Professor Hertwig from his " B " culture at various times from February 4 until May 6, 1907.

Professor Hertwig furnishes me with the following notes of the culture, which was started in the fall of 1906 and continously

overfed. "From the fourteenth to the thirty-first day of January there was enormous multiplication and feeding. The animals stopped feeding from January 31 till February 3, when feeding was resumed for a day, then there was no more feeding until February 11. On February 16 the animals were feeding enormously and there was much plasmogamy. On February 19 the culture showed many small food-free animals. After this there was heavy feeding, with increase in the size of the animals and plasmogamy until March 3. On March 11 and 13 they were feeding less. Then until March 27 there was enormous feeding, with clumping. From March 28 till April 3 there was decreased feeding.

"On April 6 the animals were in marked depression. On the eighth and eleventh day of April, they were feeding heavily again. Then, there followed a period of heavy feeding which lasted until April 25. On April 27 the animals stopped feeding and there occurred a period of marked depression, during which many of the animals died. There was no encysting. From May 1 to May 4 there was intermittent feeding. The culture died out on May 6."

The work assigned me was the accurate and detailed study of the changes present in animals taken at various stages of the culture by Professor Hertwig. These animals, with the exception of a few which were received alive, were fixed with picro-acetic and stained with boraxcarmine by Professor Hertwig, and given to me mounted in clove oil.

Descriptions and sketches were first made of the whole animals, which were then embedded in paraffin and sections cut at 7.5 μ. The conditions to which they were submitted were kept as uniform as possible.

For most of the organisms, the carmine stain was followed by either weak Delafield's hæmatoxylin, or by Heidenhain's iron hæmatoxylin. On the whole, the most satisfactory results were obtained in sections very lightly stained with Delafield's hæmatoxylin.

The material is best presented, perhaps, in groups arranged according to the months, from February to May inclusive, in which the animals were taken from the culture. In February, animals

were taken on the fourth, ninth, eighteenth and twenty-sixth. During most of this time, the culture was in depression.

The first animal, received alive February 4, was of medium size, had a few very much thickened, blunt, short pseudopodia, and showed no demarcation between cortex and medulla, and no evidence of feeding. In stained preparations, studied both before and after sectioning, the cortex and medulla were fused, the spaces of the protoplasmic meshwork were small and thickened. The nuclei were very much increased in number, and somewhat in size (13.5 to 15 μ in diameter). The chromatin was in large irregular masses, usually connected by coarser or finer bands, forming rosettes and occasionally horse-shoe shaped forms. There were neither chromidia nor pigment. There was no evidence of nuclear fusion or of division. The second animal, which had on the fourth shown in the culture the same naked eye appearances of degeneration as the first, was killed on February 9. During this interval of five days it showed evidences of recovery (regeneration or restoration).

When killed it had short thick pseudopodia. In sections, the cortex was of normal width, well marked from the medulla, with a meshwork of normal size and appearance. The protoplasmic meshwork of the medulla was condensed and in the center of the animal it was thickened by the presence of a finely granular material. The nuclei were but slightly in excess of the normal number and were confined to the medulla. They were large—15 to 16.5 μ in diameter. The chromatic substance lay either in a single round or oval mass or in several round masses of unequal size, and near the center. The nuclear framework and nuclear membranes were distinct. Occasional degenerated nuclei without chromatin were seen. Some of the nucleoli were vacuolated. There was no fusion, division or overlapping of nuclei. A few scattered chromidia lay in the medulla, but there was absence of pigment. There was no evidence of feeding. This animal, compared with the first, showed distinct evidences of regeneration after a severe depression; it had developed pseudopodia, reëstablished its cortical layer, and reduced the number of its nuclei. It was abnormal, however, in having hyperplasia, hypertrophy and degeneration of nuclei.

On February 18 two animals were killed. They were both large, long, oval in shape, with a few short, thick, blunt pseudopodia. Sections of the larger of the two showed a fairly well-defined cortex of very irregular width, and a close, condensed alveolar meshwork, which was covered with fine granular plasma, and, at the extreme border, about the whole circumference, beset with fine yellowish brown pigment granules. The cortex contained no nuclei and no chromidia. The medulla, which contained two partly degenerated *Stentors,* was of a wide alveolar meshwork, which was covered, especially about the nuclei, with finely granular material and here and there with pigment granules. There were a moderate number of round, oval and irregular chromidia, lying, usually, about nuclei, and often connected with nuclear membranes.

The nuclei, which measured from 12 to 13.5 μ. in diameter, were considerably increased in number and were hyperchromatic. The chromatin was occasionally in round, solid, central masses, but in most nuclei it was scattered in large round or oval deeply staining masses, which were sometimes separated and sometimes connected by thinner branches of chromatic material. The nuclear meshwork was often obscured by these large masses. When visible, the nuclear meshwork was wide meshed and of uneven size. There was no vacuolization of nucleoli.

The other animal showed the same changes, except the chromidia were not so numerous. It contained no food material.

The last animal of this group was killed on February 26. It was actively feeding, of medium size, oval in outline, and without pseudopodia. The cortex was well defined from the medulla, and contained several large vacuoles. In the whole animal, the nuclei appeared very numerous and chromatin rich, while throughout the medulla great quantities of large and small, round, oval and irregularly shaped chromidial masses were present.

In sections the cortex was narrow and in places fused with the medulla. The meshwork was condensed, invaded by nuclei and beset with chromidia and a few pigment granules. The medullary meshwork was close set, the walls of the alveolar spaces thick and in many places crowded with chromidia. The latter appeared partly as fine masses, partly as large round, oval, or irregularly

triangular branched masses. Here and there, also, it was in coarse vacuolated fibrils. These chromidial masses were often grouped about the nuclei and many were directly continuous with the chromatic substance of the nuclei.

The nuclei, which varied in diameter from 12 to 13.5 μ, were decidedly increased in number and were intensely hyperchromatic. There were two general types of nuclei; one, the most numerous, with the chromatic substance in large irregularly shaped masses connected by bridges of the same substance, the other, with the chromatic material scattered in small round or oval, irregular masses. The chromatic material, or nucleoli, rarely lay in the centers of the nuclei. The nuclear framework was fine and granular. The nuclear membranes were in general distinct, but, about some nuclei, they were absent in whole or part. The evidence that the chromidia arose from the nuclear chromatin was convincing, for a direct continuity between masses of intranuclear chromatin and these extranuclear chromatin bodies could be traced.

The second group comprised a series of eleven animals taken from the culture on the first, third, fourth, sixth, seventh, tenth, eleventh and thirteenth of March.

The animals of the first and third of March were of large size, feeding, with well-defined cortical layers, and rather numerous hyperchomatic nuclei of normal size. The first contained no chromidia and but little pigment. As Professor Hertwig suggested, this animal probably marked recovery after depression.

The two animals taken on March 4 were of medium size, not feeding and showed marked signs of depression, as evidenced by narrowing and condensation of the cortex, marked chromidia and pigment formation, and hyperplasia and hypertrophy of nuclei.

In sections the narrowed and thickened cortex was studded with fine refractile, yellowish brown pigment granules; the alveolar meshwork of the medulla was thickened by the presence of a homogeneous plasma and contained great numbers of chromidia and pigment granules. The nuclei varied from 10.5 to 16.5 μ in diameter, and were situated in the cortex as well as in the medulla. The chromatin was usually in the form of large irregular rosettes. The nuclear framework was distinct. About some of the nuclei

the membranes were absent in whole or in part, with disintegration of the nuclear substance (see Fig. 14). Many nuclei touched at their borders, and overlapping of nuclei was frequent. Some of the large oval nuclei were evidently examples of fusion of two nuclei (see Fig. 3).

Chromidia were abundant and were usually grouped about the nuclei, and in many instances a direct continuity between them and nuclear chromatin could be traced (see Figs. 1 and 2). They occurred as small round or oval, large, irregular, vacuolated masses and as chromidial networks.

The organism was extremely rich in pigment, which was so thickly placed over one-fourth the body as completely to obscure the protoplasm. Over the rest of the animal, the pigment was in single, scattered or in clumped, granules. It was often in an intimate relation to the chromidia. In this animal pigment granules, identical in appearance with those in the plasma, were found in the nuclei. They were scattered over the nuclear meshwork near or on the chromatic substance, and were most often found in markedly hyperchromatic nuclei from which chromidia were projecting (see Figs. 1 and 2). The animal showed no evidence of feeding.

The three animals of March 6 are amongst the most interesting of the series. They all showed narrowing of the cortex, which was invaded by nuclei; there was hyperplasia, hypertrophy and hyperchromatism of the nuclei, marked chromidia and pigment formation, with condensation and thickening of the protoplasmic meshwork. In one of these animals, which showed the largest amount of chromidia of any of the series, the thickened, narrowed nucleus-containing cortex was beset with fine highly refractile yellowish brown pigment granules. The protoplasmic meshwork of the medulla was obscured in many areas by chromidia and pigment. The chromidia were enormously abundant and, frequently, were covered and surrounded by larger and smaller clumps of pigment granules (see Figs. 6 and 7). The nuclei, which were hypertrophied (from 13.5 to 15 μ in diameter), were remarkably hyperchromatic, and chromidia were pouring out from them in large quantities (see Figs. 4 and 7). Many nuclei contained pig-

ment granules. Overlapping of neighboring nuclei (Fig. 4) was frequent, but no examples of true fusion of nuclei were observed. There were a few dissolving nuclei, with the nuclear membrane partly or completely absent and the chromatic material in small clumps. The animal was not feeding.

The animal of March 7, also not feeding, showed the same changes described for those of March 6. The pigment was even more abundant, the granules being not only scattered over the protoplasmic meshwork and about the nuclei, but often occurring in large clumps, usually in a nest of disintegrating chromidia.

Many nuclei contained pigment granules. Vacuolization of the nucleoli was common. Two other forms of nuclear degeneration were noted, shrivelling of nuclei and disintegration of the nuclear membrane, in whole or in part, with the escape of the nuclear contents into the protoplasm. Several striking examples of nuclear fusion were observed (Fig. 3).

The animals of March 10 and 11 were of medium size, not feeding and contained no chromidia; only one showed pigment. There was nuclear hyperplasia, hypertrophy and hyperchromatism. The nuclei varied from 15 to 18 μ in diameter. Touching, overlapping and apparent fusion of nuclei were common.

The last animal of the series, killed March 13, was comparatively small, and had a few short thick pseudopodia and a very thin and narrow meshed cortex. The nuclei were smaller and less numerous than in the other animals of this series. The nuclei were confined to the medulla and were not hyperchromatic. There were a moderate number of chromidia and no pigment. The animal was not feeding.

The third group consisted of a series of twelve animals killed on sixth, seventh, eighth, tenth, and eleventh of April. They were, with two exceptions, small.

Two animals were killed on April 6. The first was of medium size, contained no pigment and no food. The demarcation between medulla and cortex was distinct. The meshwork of the latter was wide and contained well marked vacuoles. The protoplasmic meshwork of the medulla was of normal size, except in the center, where it was condensed. The meshwork, especially in this central

area and about the nuclei, was covered with homogeneous or very finely granular achromatic material. A few small round or oval chromidia were about some of the nuclei. In the spaces of the protoplasmic meshwork, there were large numbers of free vacuolated nucleoli (Fig. 10), from ten to fifteen being counted in a single section.

The nuclei were markedly increased in number, but were remarkably small, measuring from 7.5 to 10.5 μ in diameter. They were often crowded together in clumps, of two, three, four or more, their membranes touching over a small area. Overlapping of nuclei was common (Figs. 8 and 9). There was no fusion of nuclei and no division figures were to be found. The nuclei varied markedly in their chromatin content, some being markedly hyper- and others markedly hypochromatic. In the former the chromatic material was usually in irregularly shaped branching rosettes and was often beset with one or more large or small vacuoles. In the latter, the chromatic substance was usually in small round or oval scattered masses, often near the nuclear membrane. In some of these hypochromatic nuclei, the chromatin lay partly within and partly without the nuclear membrane. In one such nucleus, the nuclear membrane was lacking for about one-fourth the circumference. Some nuclei contained no chromatin and others only a few fine particles.

A common change in the nucleoli was vacuolization, which affected small as well as large chromatin masses. In the large nucleoli, the vacuoles were large and ring-shaped, and frequently a number of smaller vacuoles were grouped about a large central vacuole. In many nuclei, large vacuolated nucleoli were separated from the achromatic substance and in various stages of extrusion. Some of these bodies lay just outside the nuclear membrane. It is possible that these appearances were mechanically produced by the microtome knife.

Undoubtedly the most common change by which the large vacuolated nucleoli were set free in the protoplasm was by the dissolution of the nuclear membrane and the achromatic nuclear material. All the steps of this process could be readily traced.

Another set of changes which led to the presence of large vac-

uolated masses in the protoplasm, was seen best in sections stained with iron hæmatoxylin. This change was characterized by shrivelling and condensation of the nuclei, with the accumulation of the chromatic material about the condensed nuclear membrane, and by hyaline degeneration and vacuolization of the achromatic nuclear material. The vacuolated body resulting from these changes could be distinguished from the typical, free lying, vacuolated nucleoli, by the presence in the former of a group of smaller vacuoles or of hyaline nuclear substance lying within the chromatic ring (see Figs. 17 and 18). In the typical vacuolated nucleoli, the vacuoles appeared as empty spaces in the chromatic substance. Fig. 18 shows an interesting example of hyaline degeneration of the nuclear framework.

The second animal showed the same changes described in the first. The only special difference between the two animals was the greater number of nuclei and the smaller number of free vacuolated nucleoli in the second.

This animal was infested with great numbers of small (8.25 to 11.25 μ in diameter) closely packed, hyperchromatic nuclei. They showed to a less marked degree the same changes described in the first. The animal showed no evidences of feeding.

On April 7, two medium sized animals which were fused over a portion of their cortices were killed. They both contained small food vacuoles, were without pseudopodia and had narrow but distinct cortices, the meshwork of which was small but not specially thickened. In sections they were nearly identical in structure. The protoplasmic meshwork of the medulla was relatively wide, except in the center where it was condensed. In general it was covered with finely granular, achromatic material. There was entire absence of both pigment and chromidia. The nuclei were very numerous and relatively small, varying from 8.25 to 11.25 μ in diameter. They were, for the most part, hyperchromatic, the chromatic material being usually in large masses or rosettes. Nuclei with finely divided chromatic material were uncommon. In most nuclei the chromatic material was vacuolated. Some showed the large vacuolated chromatic nucleoli described in the animals of April 6 (see Fig. 11). In general, the nuclear framework and

membranes were distinct. There were a number of nuclei in various stages of dissolution, with loss of the nuclear membrane and disintegration of the reticulum, as described in previous animals; such nuclei had vacuolated nucleoli, and this was evidently one of the processes leading to the presence of vacuolated nuclei in the plasma. Another change resulting in such free vacuolated nucleoli was the shrinkage and concentration of nuclei in association with hyaline change of the nuclear framework, as previously described. The result was the formation of hollow, spherical or bizarre shaped shells of deeply staining chromatic material, enclosing one or more large vacuolar spaces and sometimes studded with smaller vacuoles (see Figs. 19 to 25). One of these masses of vacuolated nucleolar material measured 25 μ in length (giant vacuolated nucleolus, see Fig. 25). A common shape for the smaller masses was that of a signet-ring. Some of these free vacuolated bodies were evidently due to the extrusion of a vacuolated nucleolus before disintegration of the rest of the nucleus.

The three animals of April 8 were small, but actively feeding. Two had a few short blunt pseudopodia. The cortex was narrow but well defined from the medulla. They contained no chromidia, pigment, or free vacuolated nucleoli. The nuclei were distinctly, though not greatly, increased in number, and were hyperchromatic. They varied from 9 to 13.5 μ in diameter. The nuclear chromatin was arranged either in large loosely formed rosettes or in small scattered masses. There were no evidences of nuclear division, fusion or dissolution.

On April 10, three more animals were killed. They were all actively feeding and had a few short, stout, blunt pseudopodia. Their protoplasm was of normal structure and contained neither chromidia nor pigment. The nuclei were distinctly over-numerous and were hyperchromatic. They varied widely in size (from 9 to 15 μ in diameter); the smaller type of nuclei outnumbered the large.

Of the animals killed on April 11, two agreed in all respects with those of the tenth. The third had a distinctly increased number of large (15 to 18 μ in diameter) hyperchromatic nuclei. The nucleoli were often vacuolated.

The last group consisted of ten animals which were killed between the first and the sixth of May.

Three animals killed May 1 were small, round, actively feeding, and had a few short twisted pseudopodia, a well defined and developed cortical layer containing large vacuoles. The medullary meshwork was of unequal size and beset with large and small chromidia, which often showed vacuolization. The over-numerous and vacuolated hyperchromatic nuclei varied from 10.5 to 13.5 μ in diameter. There was no evidence of fusion, division or dissolution of nuclei. Pigment was absent in two. In one animal which was especially rich in chromidia, the latter were usually covered and always surrounded by innumerable, very fine dust-like particles of chromatic material, which obscured the protoplasmic meshwork. This deeply staining "chromatin dust" was quite distinct from the highly refractile yellowish brown pigment granules, which were also present in small numbers.

The animals of May 2, 3 and 4 were in all respects similar to those of May 1.

The animal killed on May 5 was shrivelled and distorted in shape. The abnormally wide cortex contained large irregular vacuolar spaces, separated by a coarse protoplasmic framework. The outer layer of the cortex was covered over wide areas with a deeply staining homogeneous material—dissolved chromatin.

The relatively small medulla consisted of a very wide and irregular sized meshwork, the coarse strands of which were covered with finely granular achromatic material and with "chromatin dust." There was a considerable number of large and small chromidia, but no pigment. The nuclei were hyperchromatic, with a coarse reticulum and vacuolization of the nucleoli. The nuclei measured 12 μ in diameter.

The last animal of the group and of the study, for the culture died out on this day, was killed on May 6. It presented a very peculiar appearance. In the whole animal, the outlines were very irregular and no separation between cortex and medulla could be discerned. When stained with borax-carmine, the animal presented a granular non-staining outer layer and an inner irregularly shaped, granular diffusely staining mass, which formed the bulk of the organism. In sections, there was no distinction between cortex and medulla and the protoplasm was obscured by the presence of

dissolved chromatin and large vacuolated chromidia. Only an occasional nucleus could be found. One which was measured had a diameter of 10.5 μ.

The progress and interrelations of the various changes observed in these "depressed" *Actinosphæria,* the methods adopted by the animals to readjust themselves and the successive stages in the finally unsuccessful struggle can be, perhaps, presented best by contrasting the appearances met with in the various groups.

The culture sprung from *Actinosphæria* which had been caught at the beginning of winter. The animals at that time showed to a high degree the capacity for encysting, which was gradually lost, so that animals exposed to warmth no longer formed normal cysts. Some of the animals perished at this time from the formation of giant nuclei. My material sprang from animals which had survived this period.

The animals of February and March, after a prolonged and only occasionally interrupted period of overfeeding, showed profound depression, marked at times by distinct and often well marked signs of restoration. Their protoplasm was degenerated to a more or less marked degree. There was a variable amount of nuclear hyperplasia, hypertrophy and hyperchromatism. The animal of February 26 and most of those in March showed large amounts of chromidia and often pigmentation. Throughout, there was dissolution of nuclei, and, in the later animals, fusion and shrivelling of nuclei, and often vacuolization of nucleoli.

In the April group, after another period of heavy feeding, the animals were in almost all respects, except for some of the modes of nuclear degeneration, quite different from those of the previous series. The chief characteristics of the animals of April 6 and 7 were the great number of relatively small nuclei (from 7.5 to 10.5 μ and 8.25 to 11.5 μ in diameter) and the occurrence of shrinkage, hyaline degeneration, and dissolution of nuclei, with the vacuolization of nucleoli, rather than the formation of chromidia, as the modes of reduction of excessive nuclear substances.

That these degenerative methods of reduction of nuclear substances may be effective is shown by the great differences presented by the remaining animals of this group. With few exceptions they

were feeding actively; their nuclei, while usually over-numerous, were greatly reduced in number, and the general appearance of the animals was less abnormal. The nuclei were, however, both hypertrophied and hyperchromatic, due probably to the heavy over-feeding. In the last animal of the April group the nuclei reached a diameter of 18 μ.

From the eleventh to the twenty-fifth of April the culture fed heavily, and on the twenty-seventh the animals were again markedly depressed.

The animals taken on the first two days of May were feeding, the cortical layers were fairly well developed. The nuclei were of full size (10.5 to 13.5 μ in diameter), decidedly hyperchromatic and over-numerous. The nucleoli were often vacuolated, but dissolution of nuclei and free nucleoli were not seen. There were, however, enormous numbers of chromidia and much "chromatin dust." Some of the animals contained pigment.

The animals of May 3 and 4 were small, without food, and had relatively large nuclei and a somewhat smaller number of chromidia.

The animal on May 5 was shrivelled and distorted, with relatively little medulla, and the cortex contained a considerable amount of homogeneous dissolved chromatin. Otherwise it resembled those of the two previous days.

In the animal of May 6, the protoplasm was markedly degenerated and distorted, and but few nuclei could be recognized.

Reviewing the whole material, it is seen that, during the entire period covered by these observations, the animals showed not only a hyperplasia of nuclear substances, but evidences of profound changes in the structure of the protoplasm. It will be noted that typical giant nuclei were not present (the period for this having passed) and that the hypertrophied nuclei of this culture did not reach the extreme size obtained by those of Professor Hertwig's 1902 culture. I have designated as "hypertrophied nuclei" those nuclei which exceeded the extreme limit (14 μ) which he gives for normal nuclei. While there were times when the nuclear mass was reduced considerably and when the protoplasm approached its normal structure, organisms with normal nuclei in normal protoplasm were not observed; the "Kernplasmarelation" was never reëstablished, and complete recovery did not occur.

With the exception of giant nuclei, the necrosis of parts of protoplasm, fat and the transformation of all the nuclei to chromidia, all the nuclear and protoplasmic changes described by R. Hertwig ('04) in *Actinosphæria* depressed by overfeeding and prolonged culture, were observed in the series of animals described in the present communication. In regard to the absence of fat, it should be stated that most of the animals had been fixed and stained (and therefore treated with alcohol) before they were received by me.

In addition to confirming R. Hertwig's previous observations in most particulars, three new points were established in regard to *Actinosphæria* depressed by prolonged overfeeding, namely, the occurrence of pigment granules in hyperchromatic nuclei; hyaline degeneration of the nuclear reticulum leading to the formation of shrunken, vacuolated nuclei which in some respects resemble the free vacuolated nucleoli of R. Hertwig; and, finally, the fact that in *Actinosphæria* with large hyperchromatic nuclei, the reduction of nuclear mass is accomplished mainly by the extrusion of nucleolar substance and chromatin as chromidia (chromidiosis), while in animals with great numbers of relatively small nuclei, this is effected chiefly by shrinking, dissolution and various other degenerations of nuclei.

GENERAL PART.

It remains now to interpret these various nuclear and protoplasmic changes in the light of recent knowledge and to point out their relation to certain aspects of the general physiology and pathology of the cell.

In regard to the increase of the nuclear mass, it was both actual and relative. With R. Hertwig ('04) I can recognize three forms of increase of the nuclear mass, (1) the enlargement of single nuclei, (2) increase in the number of nuclei and (3) increase in the number and size of nuclei.

As previously stated, in the present series of *Actinosphæria* the time for the formation of giant nuclei was passed and the hypertrophied nuclei did not reach the size attained in Professor Hertwig's 1902 culture—21 to 35 μ. One possible explanation is that most of my measurements were made of nuclei in sections, which had been treated with xylol. Some of the nuclei exceeded 18 μ in

diameter. All of the animals, however, had an excess of nuclear material, in the shape of either a markedly excessive number of small or large nuclei or of a less marked excess of large nuclei. The protoplasm, whether of large or of small animals, always showed more or less well marked structural changes and was relatively small in proportion to the nuclear mass.

R. Hertwig has pointed out that this increase in the nuclear mass must be due to increased functional activity associated with the continuous overfeeding and the resulting overnutrition. He has suggested the same explanation for the large nuclei of carcinoma and sarcoma cells and of certain cells in inflammatory processes.

The nuclear hyperplasia in depressed *Actinosphæria* must, of course, be effected by mitosis, and it is not unlikely that the functional growth (hypertrophy) may produce in R. Hertwig's sense sufficient "Kernplasma tension" to start nuclear division. There is absolutely no evidence that the chromidia of depressed *Actinosphæria* ever give rise to new nuclei corresponding to the secondary nuclei of the Thalamorphora arising from the "Chromidialnetz."

R. Hertwig ('04) regards the occurrence of nuclear hyperplasia in depressed *Actinosphæria* as a good sign, for such animals may assimilate and multiply enormously, while most giant nuclear animals die. The division of the nuclear materials into multiple small masses would, he thinks, afford better conditions for carrying on the life processes.

The formation of chromidia and most, if not all, of the various forms of nuclear degeneration described in these animals, are to be looked upon as the results of the use of physiological means, for, to a considerable degree, the same processes occur in encysting *Actinosphæria*, in which the number of the nuclei is reduced normally to an astonishing extent. Chromidia may occur in normal animals and in the encysting process a great number of chromidia are extruded from the nuclei in the cysts, though most of the reduction in the number of nuclei occurring before encysting is accomplished by absorption of nuclei rather than by dissolution into chromidia. As in the processes leading up to encysting, so in these depressed animals, abnormally stocked with nuclear materials, the

protoplasm exerts its power of destroying the nuclei in order to reëstablish the " Kernplasmarelation."

I have stated already, that there is no evidence that the chromidia of depressed *Actinosphæria* possess a propagatory function in the sense of Schaudinn and Goldschmidt ('04), *i. e.,* they do not form new nuclei. According to Goldschmidt's ('04) interesting theory, these chromidia represent excessive somatic chromatin needed and used by the animals to assist in the active function which they are carrying on.

Against this view are the facts that the greatest number of chromidia are not present in the more actively feeding animals and that the chromidia break up into pigment. In favor of his theory, however, is the fact that, when the nuclei are large and relatively less numerous in feeding depressed *Actinosphæria,* chromidia are much more plentiful than in animals with great numbers of small nuclei. That normal *Actinosphæria* form chromidia during functional activity cannot be denied. It is possible that in depressed *Actinosphæria* the existence of two kinds of chromidia is to be recognized, one highly functioning, somatic in type, and the other formed in the efforts of the animal to reduce its surplus of nuclear substances and destined to be got rid of.

The formation of pigment from nuclear material is of great interest. R. Hertwig ('02) first suggested that the pigment of melano-sarcoma cells is formed from chromatin extruded from the nucleus in an effort of the cell to reduce its nuclear mass and thus to reorganize itself. Later ('04) he described the transformation of chromidia to pigment and recognized the possibility of the development of pigment from the nucleus, and called attention to the fact that the two agents (cellular activity and cold) which best effect relative enlargement of the nuclear mass also produce conditions most favorable for pigment formation. He referred, of course, to autochtonous iron free pigment.

R. Hertwig's pupil Roessle ('05) has traced, in the cells of melano-sarcoma, the wandering of nucleolar substance from nuclei over-rich in this material and its transformation into pigment in the protoplasm. Roessle suggested that the pigment formation of senile atrophy, probably partly physiological and partly patho-

logical, is produced in the same way as the pigment of *Actinosphærium* and showed that, as far as micro-chemical methods go, the pigments of *Actinosphærium*, melano-sarcoma and brown atrophy appear to be identical, and are iron free. Meirowitz-Grandenz ('07), studying pigment formation in the skin after exposure to the Finsen light, was able to trace the development of pigment from nucleolar substance, both in the nucleus and in the protoplasm. The same processes were evident in the *Actinosphæria* described by me.

Through the courtesy of my friend, Dr. H. Marcus, I have observed pigment granules in the nuclei of pigmented epidermal cells in his sections of Professor Brauer's *Hypogeophis rostratus* material.

The nuclear degenerations present in these *Actinosphæria* have much in common with those which occur in various pathological processes in the higher animals, and, as R. Hertwig has shown, bear a striking similarity to some of the nuclear degenerations described by Pianese in carcinoma and erroneously interpreted as parasites by various authors. This is especially true of the vacuolization of nucleoli and the hyaline degeneration of nuclei met with in the present series of animals.

The condition of the protoplasm in many of these overfed *Actinosphæria* was as abnormal as that of the nuclei. There was loss or degeneration of pseudopodia, fusion of cortex and medulla, loss of vacuoles, condensation and shrivelling of the protoplasmic meshwork, and, in R. Hertwig's previous series, necrosis of protoplasm and the presence of fat. To what causes are these changes to be assigned? In the present state of our knowledge it is impossible to explain them satisfactorily. It seems, probable, however, that the exhaustion of overwork in digestion and in breaking down nuclei, the loss of materials to the ever-growing nuclei and the intoxication from retained metabolic products are among the factors concerned.

In conclusion, I take this opportunity to express my heartiest thanks to Professor Hertwig, not only for his material, his supervision and many suggestions in this work, but for many kindnesses extended to me in his institute.

MUNICH, May 22, 1907.

EXPLANATION OF PLATES XVI–XIX.

The drawings were made with the aid of a camera lucida, under Leitz 1/12 oil immersion objective, ocular 3, tube length 160 mm.

FIGS. 1 and 2. Hyperchromatic nuclei giving off chromidia, containing pigment granules, and surrounded by chromidia and pigment. Animal of March 4.

FIG. 3. Hyperchromatic nuclei; fusion of two nuclei. Animal of March 7.

FIG. 4. Small portion of periphery of animal of March 6. Note fusion of cortex and medulla, overlapping of nuclei, chromidia and pigment.

FIG. 5. Large hyperchromatic nucleus, probably the result of fusion of two nuclei. Animal of March 3.

FIG. 6. Condensation of protoplasm, intense chromidiosis, with large central clump of pigment granules.

FIG. 7. A large nucleus surrounded by chromidia and pigment granules.

FIG. 8. Small area of medulla of animal of April 6, showing marked nuclear hyperplasia. Note that the nuclei are small and touch at their borders.

FIG. 9. Overlapping of two small nuclei. Animal of April 6.

FIG. 10. A free vacuolated nucleolus between two small nuclei. Animal of April 6.

FIGS. 11 and 12. Nuclei with vacuolated nucleoli. Animals of April 6 and 7.

FIGS. 13 and 14. Stages of dissolution of nucleoli. Animals of March 4 and April 7.

FIGS. 15 and 16. Shrunken nuclei. Animals of March 4 and April 6.

FIGS. 17 and 18. Hyaline degeneration and vacuolization of nuclei. Animals of April 6 and 7.

FIGS. 19 to 25. Types of vacuolated nucleoli lying free in the protoplasm. Animal of April 7.

All the photographs (Figs. 26–28) are taken at the same magnification = × 100.

FIG. 26. Photograph of section of normal *Actinosphærium*. Note the wide cortex and the number and distribution of the nuclei.

FIG. 27. Photograph of section of a depressed *Actinosphærium*. Note hyperplasia, hypertrophy and hyperchromatism of the nuclei, condensation of the protoplasmic meshwork and the presence of chromidia. Animal of March 6.

FIG. 28. Photograph of section of a depressed *Actinosphærium* during a period of active overfeeding. Note the numerous vacuoles and marked hyperplasia and hyperchromatism of the nuclei. Animal of April 7.

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Fig. 8.

Fig. 9.

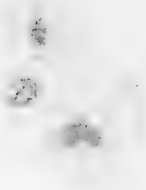

Fig. 10.

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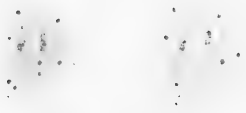

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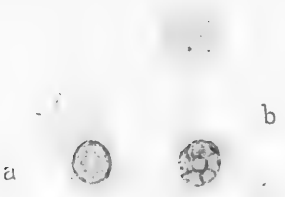

Fig. 18.

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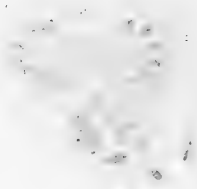

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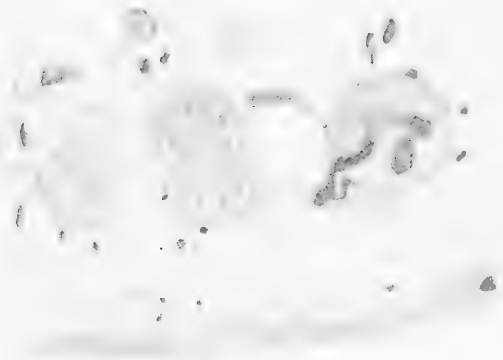

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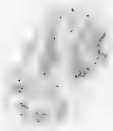

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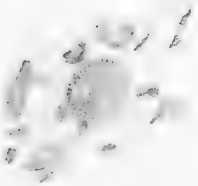

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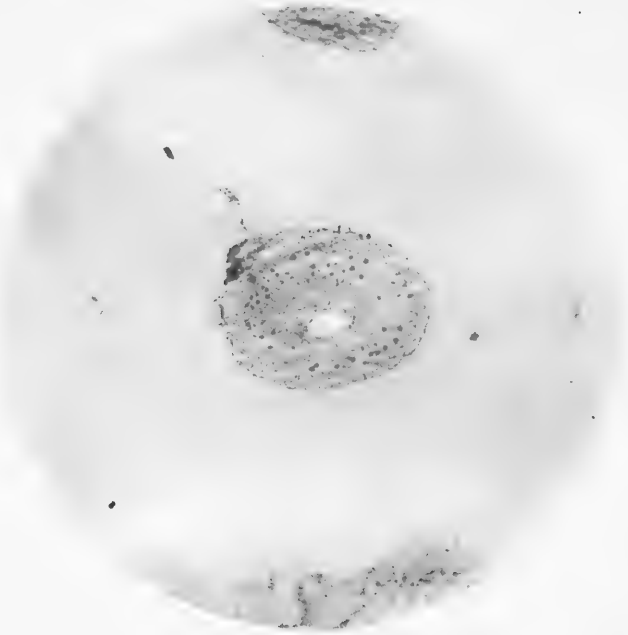

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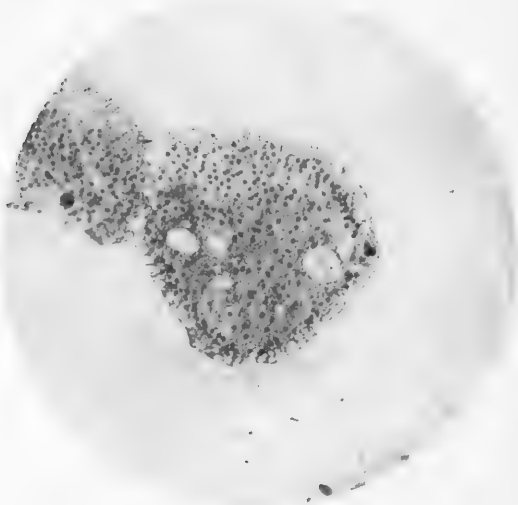

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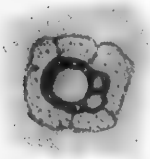

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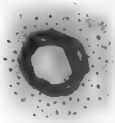

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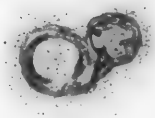

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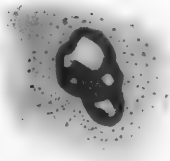

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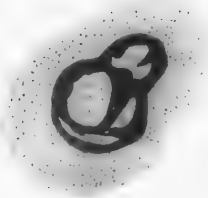

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THE OPHTHALMO-TUBERCULIN REACTION IN CATTLE.*

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von Pirquet[1] has recently made use of the local reactions sometimes elicited in individuals affected with infectious diseases when the toxin from the microörganism causing the infection is injected into the body. He has applied the phenomenon to tubercular infections and has found that when tuberculin is introduced into the dermis that a local reaction results in tuberculous subjects and no reaction of any consequence in the non-tubercular. This local cutaneous reaction lasts at least eight days. von Pirquet has applied this test in infants and children and finds that it is of diagnostic value especially in the beginning of the disease.

Vallée[2] has applied the cutaneous tuberculin test to cattle, horses and guinea-pigs. He finds that the reaction is positive in the majority of tuberculous animals and conversely does not occur in normal animals. It is not within the province of this paper to consider in detail the cutaneous reaction for tuberculosis. This subject will form the basis of a later communication.

Calmette[3] has made use of the foregoing principle, but has modified the method of applying the test. He instills the tuberculin into the eye instead of into the skin. He finds in cases of tuberculosis, after six to sixteen hours, that the conjunctiva, particularly

* Received for publication November 1, 1907.

[1] von Pirquet, *Ber. klin. Woch.*, 1907, xliv, 644.

[2] Vallée, *Compt. rend. de l'Academie des sciences*, 1907, cxliv, 1383.

[3] Calmette, *Presse médicale*, 1907, xv, 388, 443.

the palpebral conjunctiva, becomes markedly congested, that there is lachrymosis and finally the whole conjunctiva is covered with a serofibrinous exudate. In the non-tubercular there may be a slight conjunctivitis, but usually there is no reaction whatever.

Olmer and Terras[4] have found that the ophthalmo-tuberculin test in man is more accurate than the cutaneous test. They obtained some conflicting results, however, as in a few cases there was a reaction in non-tuberculous individuals whereas in a small proportion of those clinically showing tuberculosis there was no reaction.

In the light of the recent results which have been obtained by the use of tuberculin in the eye for the diagnosis of tuberculosis in infants and to a limited extent in adults, it has seemed possible that the ophthalmo-tuberculin test may be applied successfully to cattle. It is a fact, disputed by some, that bovine tuberculosis can be transmitted to man and especially to infants. This transmission takes place through the milk of tuberculous dairy cattle. It is important that an easily applied and accurate means for the diagnosis of tuberculosis in cattle be available in order that dairy herds be kept free of tubercular animals. Animals showing the least suggestion of tubercular infection should be isolated and if the diagnosis proves correct destroyed. It must be borne in mind that the tuberculin test as it is ordinarily made has many objectionable features.

With a view of investigating the efficiency of the ophthalmo-tuberculin reaction in cattle we undertook a series of experiments.

DESCRIPTION OF EXPERIMENTS.

Cattle.—The cattle used in these tests were divided into three classes as follows: Class A, five animals which reacted to the tuberculin test in February, 1907; Class B, five animals which reacted to the tuberculin test in September, 1907; Class C, twenty animals which were used as *control* and to which the tuberculin test was applied in November, 1906, no reactions resulting.

Class A.—These animals were fancy cattle and appeared to be in the best of condition with the exception of No. 2A, which was beginning to show clinical signs of tuberculosis.

[4] Olmer and Terras, *Presse médicale*, 1907, xv, 593.

Class B.—These five animals were from a herd of thirty-four dairy cattle, twenty-seven of which reacted to the tuberculin test. They are in the advanced stages of tuberculosis. *Bacillus tuberculosis* was found in the fæces of No. 2B.

Class C.—These animals were from a large dairy herd, nineteen of which had been repeatedly tested with tuberculin and had shown no reaction. Cow No. 2C is a two-year-old Jersey heifer and has a temperature (103°–104° F.) which is continually too high to be made use of in a usual tuberculin test. This cow will be tested as soon as possible.

TUBERCULAR CATTLE.

Class A.		*Class B.*	
1A.	Polled Angus	1B.	Jersey
2A.	Jersey	2B.	Jersey
3A.	Short Horn	3B.	Jersey
4A.	Short Horn	4B.	Jersey
5A.	Short Horn	5B.	Jersey

NON-TUBERCULAR CATTLE. CONTROLS.

Class C.		*Class C.*	
1C.	Grade Short Horn	11C.	Guernsey
2C.	Jersey (heifer)	12C.	Guernsey
3C.	Grade Short Horn	13C.	Jersey
4C.	Grade Short Horn	14C.	Jersey
5C.	Grade Short Horn	15C.	Red Polled
6C.	Dutch Belt	16C.	Red Polled
7C.	Jersey	17C.	Grade Red Polled
8C.	Jersey	18C.	Red Polled
9C.	Jersey	19C.	Grade Holstein
10C.	Jersey	20C.	Red Polled

Technique.—The method first tried was the administration of the tuberculin as prepared by Calmette. A one per cent. solution was made by dissolving the precipitate, obtained by treating tuberculin with absolute alcohol, in sterile water. The solution was sterilized by steam at 100° C. One-tenth (0.1) to one-fifth (0.2) cubic centimeter of the above solution was placed in the conjunctival sac of each cow. No results were obtained by the use of this method. There is a possibility that the amounts used were too small.

The tuberculin which proved to give the most successful results was procured from the Bureau of Animal Industry, United States Department of Agriculture. It was used full strength and twenty-

five-hundredths (.25 c.c.) of a cubic centimeter was placed in the conjunctival sac of each cow with a sterile eye dropper. The right eye was used, its condition being noted and compared with the left at the time of injection. Accurate data were recorded in regard to the temperature before and after the instillation of the tuberculin into the eye.

Reaction.—In the right eye, beginning from six to eight hours after the instillation of the tuberculin (.25 c.c.) the lids appear slightly swollen. Photophobia and lachrymosis are present. Within from sixteen to eighteen hours there appears upon the conjunctiva, a thin layer of whitish gray membrane. This is particularly marked on the bulbar conjunctiva covering the sclera and on the membrana nictitans which is reddened. Thin films of this membrane are constantly being washed down over the cornea, where they appear as movable opacities, passing downward to the lower lid. The eye-lashes are agglutinated with greyish yellow exudate. The episcleral blood vessels are dilated. From the inner canthus is a discharge of straw-colored exudate, which dries to form crusts of dried exudate below the inner canthus. The left eye is perfectly normal.

Statement of Results.—The reaction above described was found in all the tubercular cattle, *i. e.,* Classes A and B, and a slight reaction in Cow 2C. It will be recalled that 2C had not been tested with tuberculin regularly on account of her continued high temperature. The fibrinous exudate in this case was very slight. Class A showed the most profuse exudate and the most typical reaction. These cattle had not been tested with tuberculin since February, 1907. This point should be noted: Class B showed the reaction, but not in so typical fashion as Class A. These cattle were tested with tuberculin only four weeks before the ophthalmo-tuberculin test was administered. This point should also be noted: All the control cattle, *i. e.,* Class C, with the exception of 2C, which showed a slight reaction, gave no reaction whatever. Cows 1C to 7C inclusive, were tested twice and showed no reactions. No rise in temperature or other constitutional disturbance was noted in any of the cattle.

CONCLUSIONS AND SUMMARY.

The conclusions to be deduced from this brief series of experiments are as follows:

1. The ophthalmo-tuberculin reaction is of some value for diagnosis of tuberculosis in cattle. A characteristic conjunctivitis with fibrinous exudation coming on from six to eight hours, reaching a maximum in from sixteen to twenty-four hours and disappearing in forty-eight hours, is noted in tubercular animals.

2. The reaction is more pronounced in those animals which have not been recently tested with tuberculin. With this reaction as with the usual tuberculin test one injection and reaction probably inhibit a second reaction during a period from six weeks to a year. The ordinary tuberculin test does not seem to interfere to any great extent with the ophthalmo-tuberculin test at least within four weeks. Class B, though recently tested, showed the reaction, although not to the extent of Class A, which was tested some time ago. The tuberculin test occasionally prevents absolutely a second reaction, and usually no second reaction occurs within six weeks to a year, as before stated.

3. In cattle recently tested with tuberculin by the subcutaneous method the ophthalmo-tuberculin reaction is only slightly reduced in its intensity. The ophthalmic test may possibly serve as a means of diagnosis of tuberculosis in cattle which have been tested with tuberculin by the ordinary method and will not react a second time, or where tuberculin has been injected into cattle in order that they may clear a second test.

Another possibility must not be overlooked in this connection. It is well known that animals which have a slight tubercular infection often show a very marked and typical reaction to the tuberculin test as it is usually made, and those animals which show themselves clinically to be in the advanced stages of tuberculosis often show only a very slight reaction. The animals in Class B are in the advanced stages of tuberculosis and this fact might perhaps account for the lower intensity of the reaction. The exact cause of the lower reaction in these cattle can only be determined by further experimentation.

4. No constitutional disturbance being noticed in any of the cattle

tested, that is, no rise in temperature, loss of appetite or falling off in the production of milk, it is evident that the instillation of tuberculin into the eye does not produce the general reaction which attends in some cases the subcutaneous injection of tuberculin, and is therefore decidedly advantageous. The exudate disappears and leaves the eye perfectly normal in forty-eight hours after injection.

5. If the ophthalmo-tuberculin test proves as efficacious as the foregoing experiments seem to indicate we have in it a comparatively rapid and easy means of diagnosing tuberculosis in cattle. Such being the case the method cannot fail to come into general use superseding the present laborious method of applying the test. Cattle can be injected and then inspected sixteen to twenty-four hours afterward.

There are many problems in connection with the reaction which must necessarily be studied. The following are a few of the propositions:

1. Is it possible to tell by the reaction how far the tubercular process has progressed in the body?

2. Is there any relationship between the intensity of the reaction and the number and severity of the tubercular lesions?

3. Will the test prove to be more accurate than the ordinary tuberculin test which is said to reveal all but four per cent. of the cases?

4. Will the test reveal tuberculosis after a subcutaneous tuberculin injection?

5. Will animals react a second time if the first tuberculin is placed in the eye and the second given by the ordinary subcutaneous method and conversely?

These and many more points must be thoroughly investigated before the efficiency of the test is proven.

The opththalmo-tuberculin test will be repeated on other cattle, and also on the cattle used in these experiments. Experiments are being made to determine the efficiency of the cutaneous tuberculin reaction in cattle. We wish in this paper to give only the preliminary findings.

AN INQUIRY INTO SOME MECHANICAL FACTORS IN THE PRODUCTION OF LYMPHOCYTOSIS.[1]

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The paper that follows deals with an attempt to separate out, and study, some of the factors which produce the clinical feature of lymphocytosis. The truth of Ehrlich's (1) doctrine that an absolute lymphocytosis is due, apart from changes in the productive activity of the lymphoid tissue, to a flushing out of the cells through increase in lymph-flow, though supported by clinical evidence, has never been proved. Indeed, there have been few attempts to come directly by experiment to the forces determining lymphocytosis; this, too, despite manifold labours to plot the fluctuation of the blood-content in lymphocytes caused by divers physiological and pathological conditions.

For the experiments here detailed the cell-output by way of the thoracic duct was utilized. Of late this path to the blood for lymphocytes has been held to be of comparatively little importance. The recognition of lymphoid centers in the bone-marrow, the study of the abundant lymphadenoid tissue of the digestive tract, the observations for a direct passage of the lymphocytes into the blood-vessels, and the realization that the lymph has important functions of its own, have tended to this conclusion, as have the many assertions that the lymph of the thoracic duct carries few lymphocytes in comparison with the blood's needs. Nowadays, as Delamere (2) says, we hold that "the lymphocytes are the casual guests of the lymph." They are supposed to be formed in the lymph-glands and the lymphadenoid tissues in general, the spleen, and the bone-marrow, with direct entrance through the vessel-walls as a frequent way by which they reach the blood.

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication November 18, 1907.

Nevertheless, recent experimental evidence points to the thoracic duct as the chief way to the circulation for the lymphocyte. Biedl and v. Decastello (3), working on dogs, produced fistula of the thoracic duct, and found that the lymphocytes in the blood decreased between 18 per cent. and 62 per cent. in absolute number; suspecting accessory channels, they carefully ligated the lymphatics on both sides of the neck, and obtained in the one animal so treated a diminution in the lymphocytes of 79 per cent. Selinoff (4), in a study of the blood of 18 dogs with fistula of the thoracic duct, noted an even more marked decrease. Thus, for example, in two of his cases there were, respectively, 1,800 and 2,000 lymphocytes per cubic millimeter in the blood just prior to operation, and on the fifth day thereafter, in the first case, only 100 such cells, and on the seventh day, in the second case, only 200 such cells per cubic millimeter. He made certain by controls that these results could not be laid to the effects of the operation itself. Crescenzi (5) observed the blood after splenectomy and the establishment of a fistula of the thoracic duct in the same animals. He obtained a decrease in the lymphocytes of from four fifths to ten elevenths of their number. Parodi (6) following Crescenzi and Selinoff, came to the conclusion that, in dogs, fistula of the thoracic duct, with or without splenectomy, brings about a diminution in the quantity of lymphocytes in circulation. Unfortunately he omits the figures supporting this. Yet those cited above seem convincing when one considers the direct anastomoses known to exist between the lymphatics and the blood-vessels (Lippi, Boddaert, Leaf (7)), and the undoubted migration of some lymph-cells directly through tissues into the blood-stream. True, the diminution that the figures represent is transitory; but this only emphasizes the presence of a compensatory mechanism that must mask, to an extent, the full effect of the fistula.

It has been objected that the number of cells furnished to the blood through the thoracic duct is quite inadequate to maintain the percentage of lymphocytes seen. But the important element in such calculations,—the term of existence in the circulation of the individual cell,—is not known. The number coursing through the thoracic duct (from 2,000 to 7,000 in the cubic millimeter of dog's

lymph—Winternitz (8)) may be quite adequate, as Biedl and v. Decastello are at pains to show, for the needs of the circulation.

Whether or not the thoracic duct furnish the majority of the lymphocytes to the blood, as above indicated, the system of which it is the outlet,—a more or less completely "closed" system with one principal duct,—is the part of the hæmatopoietic apparatus most accessible to direct investigation as regards variation in cell-output.

The cell-content of the lymph has been comparatively little observed, and this mostly before the discovery of the bone-marrow as a blood-forming organ, and hence before the study of the blood-cells in its modern sense. Following Virchow's (9) demonstration of the identity of the "small mononuclear" with certain elements found in lymph-glands, several observers showed these cells to be more numerous in the lymph coursing from a gland than in that coming to it (Heydfelder, Brucke, Frey (10)). Löwit (11), counting the elements from the thoracic duct of the rabbit, obtained a great increase in them by the administration of substances causing blood-leucocytosis,—a result which has since drawn some criticism. Winternitz took lymph from the vessels of the dog's thigh, following the injection of turpentine into the corresponding foot, and came to the conclusion that with inflammation of a part the cell-content of the lymph coming from it is increased, and the majority of the cells becomes one of polymorphonuclear neutrophiles. Goodall and Paton (12), during an investigation on digestive leukocytosis, made counts from several points in the lymphatic system, but with very irregular results, except for evidence that pointed to a sedimentation of the cells in the receptaculum chyli. Recently Forgeot (13) has examined the lymph of ruminants as it escaped from a thoracic duct fistula, with no constant findings, however, beyond that of a greater cell-content in the fluid from young individuals. There have been no adequate researches on the cell-content of the lymph under varying physiological conditions. Yet, needless to say, the quantity of cells in the lymph represents one side of the activity of the lymphadenoid tissue; and variations in this quantity, in addition to their value as an index to the state of that tissue, have a bearing on clinical lymphocytosis and lymphopænia, and, ultimately, on the meaning of the lymphocyte.

To obtain a count that represents the average number of elements in the lymph flowing at a certain time, one should obtain a thorough mixture of a considerable quantity of it. For, by reason of the inconstancy of lymph-flow, as tending to a sedimentation of cells, and the anatomical arrangement of the lymphatics, which prevents the mingling of successive portions of the fluid, it follows that the individual drops, as they come from the vessel, must differ much in cell-quantity. Nevertheless one finds that of the few authors who have interested themselves in the cell-content of the lymph, practically all have taken their counts from the single drop,—which accounts for much of the irregularity in their results. Those above cited did this, except Forgeot who, in his work on ruminants, utilized one quarter of a cubic centimeter,—an extremely small quantity, considering the large size of the animals on which he experimented. Dastre, Henri, and Stodel (14), in an investigation of the effect of peptone on the cells, allowed the fluid to collect in the ligated end of the thoracic duct, or in the subclavian vein, there mixing it "by light, inconstant pressure." But, in addition to awkwardness, this measure gives the opportunity for large error in successive counts. I have employed, for the work here reported, a means whereby could be utilized a quantity of lymph sufficient to insure a result representing the average cell-content of the lymph at that time. Since dogs were the animals used, and the thoracic duct the point of collection, several cubic centimeters were deemed necessary for each test, owing to the large lymph-flow (64 c.c. per kilo per diem, or, in a dog of 18 kilo, 4 c.c. in every 5 minutes,—Heidenhain (15)). The following technic was adopted:

Three cubic centimeters of lymph are allowed to flow into a tube that contains an equal quantity of a 4 per cent. solution of sodium citrate in 0.8 per cent. salt solution,—a mixture suggested by Wright (16) for the preservation of blood unclotted and with its elements intact.[2] The tubes for this purpose are 9 mm. in bore, and are graduated accurately to 3 c.c. and 6 c.c. By reason of the

[2] Wright recommends the use of 1 part of the sodium citrate solution to 5 parts of human blood. So little of the solution will not keep the dog's lymph from clotting. A mixture of the two in equal parts is just sufficient to serve the purpose.

narrow caliber it is easy to control an error in volume to within the limits of a single drop, and, with care in the preliminary introduction of the sodium citrate solution, to confine this error almost entirely to the amount of lymph added. But let us suppose in the combined bulk of 6 c.c. (or about 90 drops) the largest tolerable error,—that of one drop in the quantity of sodium citrate solution added, and of one drop in the total mixture. The extremes here possible are, 44 drops sodium citrate solution to 47 drops lymph, and 46 drops sodium citrate solution to 43 drops lymph; or 47/91 of lymph in the first and 43/89 in the second mixture, a variation from the supposed ratio (45/90) of slightly over 3 per cent., or an error of 150 cells in a count of 5,000. This may be neglected.

FIG. 1.

The lymph as it falls from the cannula into the sodium citrate solution is mixed with this by means of a fine wire, and, when the 3 cubic centimeters have been obtained, a few glass beads are introduced to aid in the distribution of the cells on shaking, and the tube stoppered preliminary to this. For stoppering a piece of glass rod with a flange of rubber is used, a capillary opening through the center of the rod permitting the escape of air so that the stopper may be pushed flush with the fluid. (*Vide* sketch in cross-section.) The closed tube is shaken for 5 minutes; a portion of its contents drawn into a "melangeur" with 1/100 its bulk of a saturated aqueous solution of methyl violet (5B); this in turn shaken for three minutes, and a count made as for blood. The lymph is thus counted in about 1/2 concentration (99 parts lymph to 101 parts diluting fluid). Leukocytes take the violet stain, whereas erythrocytes do not.

To test the method counts were made at intervals from the same tube of lymph-sodium-citrate mixture.

Thus at the end of seven hours the leukocyte-count coincides, practically, with that first taken. The cells are undergoing degenerative changes at that time, yet may be easily enumerated. But it is necessary, for this, that the tube be agitated at least once per hour. Otherwise, the cells sediment, cohere, and cannot be easily distributed again.

Dog.	Tube No.	Time between Counts.	R. b. c. per cmm. Lymph.		W. b. c per cmm. Lymph.	
			At First Count.	At Second Count.	At First Count.	At Second Count.
Jl	V	2 hours	not tested		3,880	4,240
El	XII	2½ hours	440	440	11,740	12,360
Nl	VII	2½ "	39,840	22,240	8,980	10,080
Ol	VI	3 hours	3,940	1,810	not tested	
Ml	V	3½ hours	5,080	3,720	3,300	3,660
Ll	II	4 hours	18,560	10,960	11,920	11,180
Gl	III	5 "	not tested		13,120	13,440
Kl	I	5 "	1,800	1,000	2,400	2,120
Ol	I	5 "	not tested		6,760	6,900
Pl	I	5 "	3,120	1,180	2,760	2,800
Jl	III	7 "	14,720	12,800	4,060	3,800
El	X	18 "	not tested		7,040	6,440

It is different with the erythrocytes. These seem to disappear rapidly in the mixing fluid, notwithstanding the fact that, to judge from the unchanged or slightly crenated shape of such corpuscles as remain, and the absence of shadows, this destruction is not due to osmotic changes. Since the lymph of nearly all dogs contains red cells, an idea of their quantity, granting them to be due to blood-contamination, is important in work having to do with the white cells of the lymph, since it furnishes an index to the number of leukocytes also brought in from the blood. But if, on the meeting of the lymph with the sodium citrate solution, many of the red cells go immediately to pieces, this index is destroyed; and one might have in the specimen many white cells from the blood without trace of the contamination, so far as red cells are concerned. This possible source of error was tested for as follows:

Dog Cl.—Blood taken during the experiment had 8,600,000 r.b.c. and 15,800 w.b.c. per cmm., of which last 70 per cent. proved to be polymorphonuclear neutrophiles, giving thus 9,660 such cells in the cmm. of blood, or about 1 to every 890 r.b.c. The lymph at this time contained on count from the sodium citrate mixture 1,545 w.b.c. and 5,685 r.b.c. per cmm. Calculating from the ratio existing in the blood, one should have 6 polymorphonuclear neutrophiles introduced with these red cells. A differential count of the lymph obtained at the time will test this supposition, since the normal lymph of the dog contains extremely few polymorphonuclear neutrophiles of its own (Delamere, Biedl and v. Decastello). As a matter of fact, in this instance the lymph showed out of 384 cells counted 2 polymorphonuclear neutrophiles, or 8 in the 1,545 w.b.c. of a cmm. So the number of red cells found in the lymph seemed to be practically that introduced from the blood. Several controls of this type gave the same result.

Despite this proof that the sodium citrate solution is, for *prompt* enumeration, a medium wherewith can be obtained an approximate estimate of the red cells, no lymph was admitted for the white count, of which the erythrocyte content was large enough to suggest that the accompanying leukocyte contamination might influence appreciably the results. The number of polymorphonuclear neutrophiles found in the lymphs used formed, as above shown, an additional indication of the amount of this contamination.

In the work here detailed the lymph's content in white cells is alone dealt with.

The effects of muscular exertion (struggle) on the cell-content were first observed. Adult dogs were employed. They were given 0.5 centigramme of morphia sulphate per kilo of body-weight (Nolf (17)) 1 hour before the operation, and chloroform when necessary during it. For from 24 to 48 hours prior to the operation no food was allowed the animals, though they were provided with water. The thoracic duct was bared in the usual way, a cannula introduced into it just above its entrance to the vein, and the entrance tied off together with such lymphatics from the neck as joined the thoracic duct, with the result that the fluid brought by the thoracic duct proper was alone collected. During this procedure very little blood, at most 3 to 4 cubic centimeters, was lost. The cannula used was of narrow bore, as recommended by Nolf, since the rapid flow through such a tube allows little opportunity for clotting. Nevertheless, in about half the experiments a delicate clot formed within the cannula in the course of some minutes, so that the occasional use of a fine hooked wire was required to keep the bore clean. No tubes of lymph were counted in which the least clotting appeared, nor were any used regarding which it seemed possible that clots in the cannula might have altered the gross cell-number. The presence or absence of clotting is mentioned in the report of the individual experiments.

It was first necessary to observe the variations in the lymph's cell-content under the circumstances above outlined and with the animal quiet, since these circumstances do not imply an absence of changes that might affect the cell-content. The shunting of the lymph from the body, following the opening of the thoracic duct,

produces marked alterations in the body-fluids (the blood, for example, concentrating, the lymph becoming less in amount and of different character). This might affect the lymph's cell-content. Furthermore, as the experiment progresses, the effect of the morphia wears off and chloroform must be pressed into service. Other unavoidable changes might be cited. The behavior of the cell-content under these influences must be reckoned with before one can proceed.

Accordingly, in animals carefully anæsthetized to a state of quiet, though not of complete muscular relaxation, a lymph-fistula was established, and specimens of lymph collected at short intervals during the next several hours. The time required to obtain each portion of three cubic centimeters was carefully noted as indicating the rate of lymph-flow at that period. Full records were also kept of all restlessness of the animal, of the incidents of anæsthesia, etc. When important, these are included in the description of the experiments.

Experiment I.—Mongrel collie; male; wt. 13 kilo. The ainmal was given no food for 48 hours previous to experiment. Throughout the time of lymph-collection it was quiet, except for occasional tremors in the limbs. Lymph very slightly opalescent; no clotting noted in cannula or tubes. Seven tubes were taken, and immediate estimate made of their content in white cells.

The dog at autopsy proved to have been sound, except for a chronic thickening of one segment of the tricuspid valve; no evidences of functional insufficiency of this valve.

The results are best expressed in the form of a chart. (Chart 1.)

Of the three curves on this chart one represents the rate of lymph-flow, a second the number of cells per cubic millimeter of lymph, and the third (which is the resultant of these two) the total cell-output in a given period.

It will be observed that throughout the course of the experiment the lymph-flow gradually but steadily lessened in rapidity, and hence in amount voided. This is, of course, no new finding (Lassar (18), Heidenhain and others). The number of cells per cubic centimeter of lymph, the "cell-concentration," as it will henceforth be termed, remained nearly constant, sinking slightly at the last count. It follows from these findings that the total cell-output underwent a marked gradual diminution.

To test these results two similar experiments were done.

Experiment II.—Collie; male; wt. 23 kilo. The animal was given no food for 48 hours previous to operation. Lymph began to escape from the thoracic duct (which had been ligated 1 minute before opening) 15 minutes prior to the collection of the first tube for cell-estimation. It was very slightly opalescent; no clotting was observed. Counts taken in each case immediately after collection. Eight tubes were obtained at half-hour intervals. Throughout, the animal was absolutely quiet. (See Chart 2.)

Dog killed and autopsied; it proved to have been quite healthy.

Here the same lessening of the lymph-flow is noted. The cell-concentration, markedly greater than in Experiment I, fluctuated

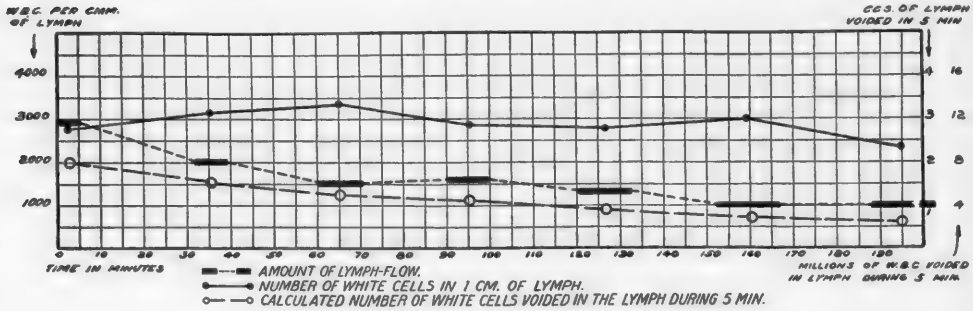
W.B.C. PER CMM. OF LYMPH
CC'S. OF LYMPH VOIDED IN 5 MIN
4000
3000
2000
1000
4
3
2
1
16
12
8
4
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
TIME IN MINUTES
MILLIONS OF W.B.C. VOIDED IN LYMPH DURING 5 MIN.
AMOUNT OF LYMPH-FLOW.
NUMBER OF WHITE CELLS IN 1 CM. OF LYMPH.
CALCULATED NUMBER OF WHITE CELLS VOIDED IN THE LYMPH DURING 5 MIN.

CHART 1.

CHART 1. The height above the base-line of the curve representing amount of lymph-flow indicates the number of cubic centimeters voided through the thoracic duct in a given time; and the black rectangles show the period required to collect the three cubic centimeters of lymph in each specimen. Thus the curve depicts in two ways the rapidity of lymph-flow.

proportionately, but in general remained constant during the first 2½ hours, after which it rose abruptly. The cell-output fell till toward the close of the experiment, when it regained nearly its former level.

Experiment III.—Skye-terrier; male; wt. 11 kilo. The fast before operation was of 24 hours duration, yet the lymph was quite chyliform throughout the period of observation. Clotting in the cannula necessitated several times the use of the hooked wire to avert blocking of the lymph-flow. The thoracic duct was ligated one half hour, and opened 15 minutes before the collection of the first tube for cell-estimation. The contents of the tubes were submitted to count in the order of their collection, but not till 3 to 4 hours after it, that is to say

at the close of the experiment proper. Seven tubes were collected at intervals in a period of 240 minutes. (See Chart 3.)

Animal normal, to judge from findings at autopsy.

A gradual drop in the rapidity of the lymph-flow occurred, similar to that in the other experiments, except for the presence of two transient fluctuations, apparently traceable to respiratory changes. The cell-concentration remained practically constant throughout the four hours, at the end of which it differed by only 100 cells per

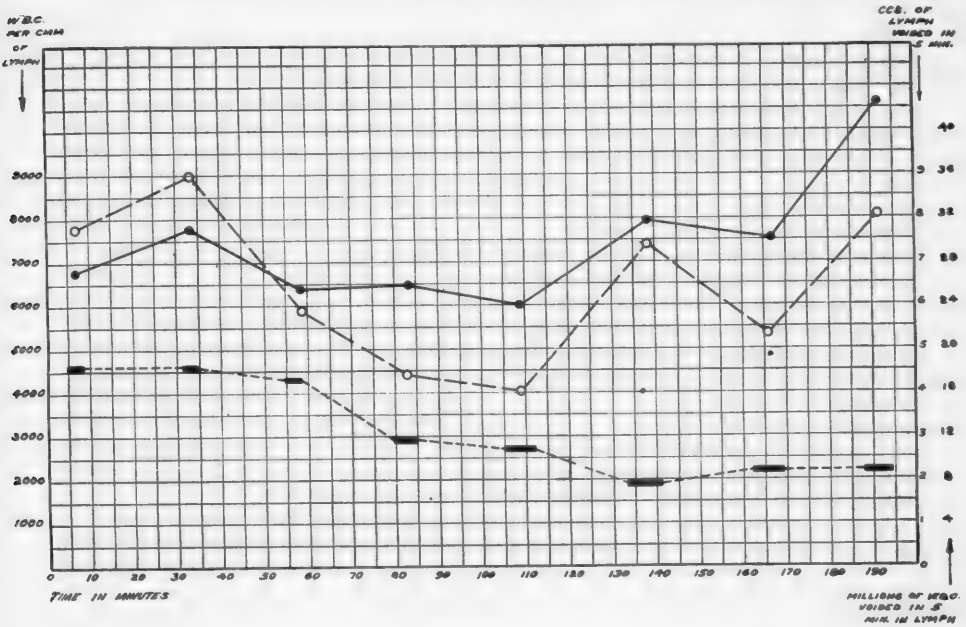

CHART 2.

cubic millimeter from that at the beginning. The total cell-output diminished, except during the fluctuations in lymph-flow above noted.

The results from the three animals form nearly a unit and are best discussed together. In all were observed:

1. A gradual decrease in the amount of lymph voided. This is no new finding.

2. A cell-concentration that varied little during the first 2½ hours of lymph-fistula. Quantitatively the variation accords with the degree of cell-concentration involved, being greatest in Experiment I, with its high cell-concentration (averaging 6,981 cells per cubic millimeter, from which there is a variation of 997 cells, or 17.6 per cent.) and least in Experiment II (in which the cell-concen-

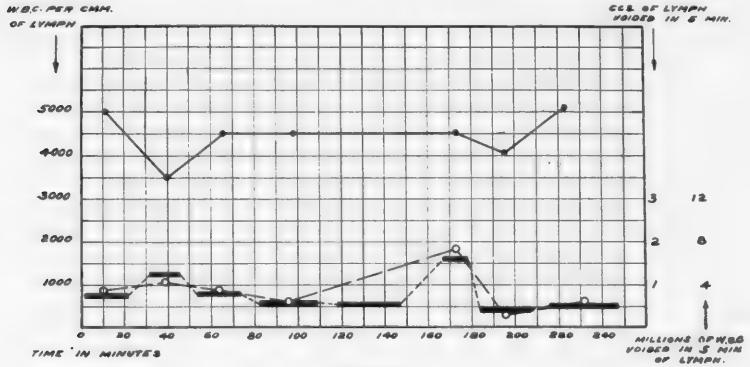

CHART 3.

tration averages 2,886 cells per cubic millimeter, and the variation is 506 cells, or 14.3 per cent.). In Experiment III the variation is 21.8 per cent.

So much for the cell-concentration during the first 2½ hours. Later, in one case it rose markedly, in another fell slightly, in the third remained unvaried. The only previous counts under conditions somewhat similar are those of Forgeot already referred to. The animals he employed (ruminants) were not anæsthetized, and his results show a variation, often of many thousand cells per cubic millimeter, in the counts from hour to hour. One understands this better on consideration of the small quantity of lymph he used in his estimations, the clotting that he frequently had to do with, and the absence of precaution to prevent struggle, which, as will later be shown, has a profound effect on the lymph's cell-concentration. Of fifteen animals on which he made observations, often over a period of more than 24 hours, the lymph in eight showed in general a tendency to lessened cell-concentration, in six, apart from large

transient variations, there was no change, and one exhibited an increase.

For our purposes it may be accepted that in the fasting dog anæsthetized with morphine and chloroform the cell-concentration of the lymph escaping from a fistula of the thoracic duct, is, during the first 2½ hours, fairly constant, when the animal is quiet and the lymph formation is suffered to take place undisturbed. The variation in cell-concentration during this period is not greater than 25 per cent.

3. The total cell-output, apart from transient fluctuations dependent on those in the lymph-output and cell-concentration, showed a decided tendency to lessen.

Here an interesting point presents itself for discussion: How is a constant cell-concentration maintained during 2½ hours under the conditions of a slowing stream of lymph, and a diminishing total cell-output? The explanation is not evident.

According to Ehrlich, the lymph-cells, on their maturation, are caught up by the lymph-stream and transported passively into the blood; or, as he puts it in another connection, "one is obliged to conclude that a lymphocytosis occurs, when, in response to an increased circulation of lymph in a greater or less extensive lymphatic region, more elements are mechanically forced from the lymph-glands."[3] Can one suppose that here the gradually lessening cell-output occurs because the lymph, through slowing, is rendered unable to force from the glands, and transport, its usual quota of cells? Or do the lymph-cells sediment along the course of the vessels in which they travel? Goodall and Paton, on the basis of counts from the receptaculum chyli, hold that some sedimentation in this reservoir is normal. Or do the lymph-glands, under the circumstances of the experiment, fail progressively in the maturation of cells? Any one of these happenings might explain the case.

Struggle, as will later be shown, increased promptly and markedly the cell-concentration of the lymph, although previously, under conditions of quiet, it had been lessening gradually. Thus it is

[3] The observations for an "active lymphocytosis" (Almkvist (19), Wolff and v. Torday (20), Proscher (21)) do not affect this conclusion, since they are concerned, not with lymphocytosis of the blood, but with the emigration of lymphocytes into the tissues and body-cavities.

shown that the glands are not lacking in cells fit for output. So the third hypothesis falls to the ground. One is left to explain in mechanical ways the constant cell-concentration in a lymph diminishing progressively in amount voided. One may suppose that the slowed current is not capable of transporting all of the many cells ready for it, and that, of those it picks up, some "sediment" on the way to the thoracic duct. The late rise in cell-content in Experiment II might, perhaps, be cited as an instance in which, despite these factors, the lymph became crowded with cells from the accumulation of those ready for it. In any event, the fact that the cell-concentration remains so long unchanged is surprising; one would expect to find immediately such variations as showed themselves only after several hours. Yet that the results are not (as might be supposed from Forgeot's work) examples of coincidence, is shown by the charts illustrating the effects of muscular exertion and of lymphagogue action. In these, despite varying cell-concentrations with varying physiological states, the same tendency to a constant cell-concentration is noted to occur hand in hand with a diminishing lymph-flow.

Discussion on the difference in average cell-output of the individuals will be reserved at this point.

With these results as a control, the effects of muscular exertion were taken up. It has been long known that this greatly accelerates the flow of lymph (Genersich, Lassar, Cohnheim (22)), but there have been no observations of its effect on the lymph's cell-content. A priori, on the theory that an increased output of lymphocytes is due, apart from special activity of the cell-forming tissues, to the flushing action of increased lymph-flow, one should find a transient increase in cells, traceable partly to those elements washed from the lymph-glands, and partly to those caught up from the channels by the swift current. But the existence of this increase, its amount, its duration, its effect on the blood, are all matters of conjecture.

In the experiments that follow, the animals were treated as those previously, except that they were at intervals made to struggle. Since morphia sufficed for the most part as anæsthetic, this was easily accomplished by giving a strong whiff of chloroform, or by tweaking the skin, or by an abrupt noise.

Experiment IV.—Irish setter; male; wt. 20 kilo; no food for 24 hours prior to operation. The thoracic duct was ligated, and a cannula introduced into it 5 minutes before the collection of lymph-specimens began. The lymph was slightly opalescent at first, later it was clear and yellowish; no clotting in cannula or tubes. The periods of struggle are noted on the chart. The cell-counts were made in the order in which the tubes were obtained, and between three and four hours after the collection of each one.

No autopsy done.

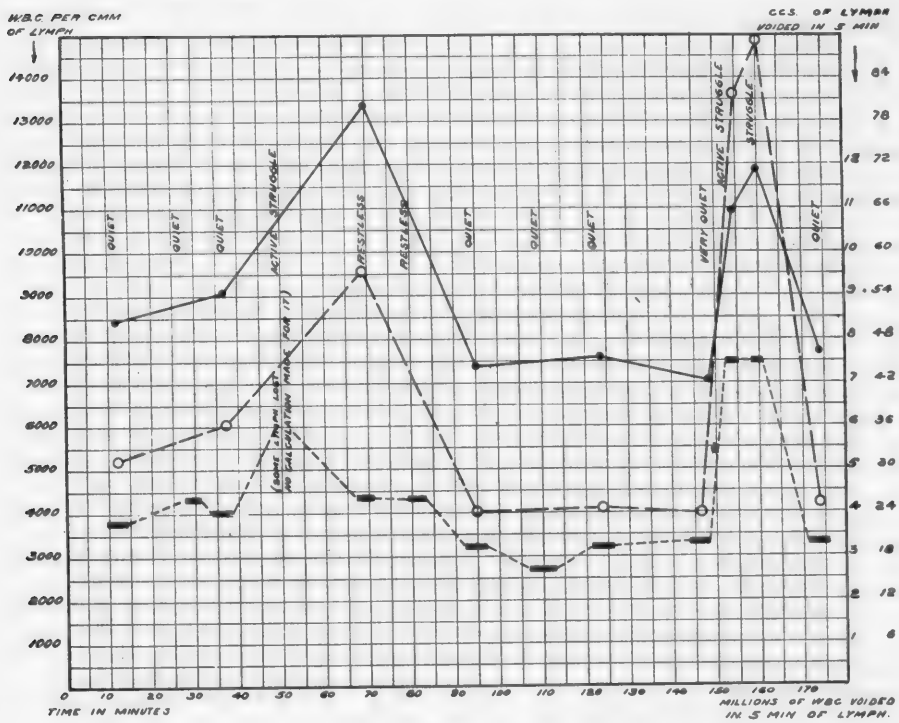

CHART 4.

In this instance struggle was twice induced. Each time the lymph-flow quickened abruptly and considerably, but with the return of quiet sank to below its former level. Each time, too, the cell-concentration became much greater. Thus the cell-output as a whole was multiplied. Indeed, it was necessary in this, and in the succeeding charts, to flatten the curve representing the cell-output.

With the return of quiet the cell-concentration and cell-output sank to slightly below their level previous to the exertion.

The next experiment was similar, but the lymph-specimens were collected at very short intervals.

Experiment V.—Bull-terrier; male; wt. 18 kilo; no food for 24 hours before operation. The duct was ligated, and opened, 4 minutes before the beginning of lymph-collection. Lymph at first slightly opalescent, yellowish and clear toward close; no clotting in tubes or cannula. The cell-estimations were made in the order of tube-collection, and 2 to 3 hours following this.

Animal at autopsy proved to have been sound. A tape-worm and several round-worms were found in the intestine.

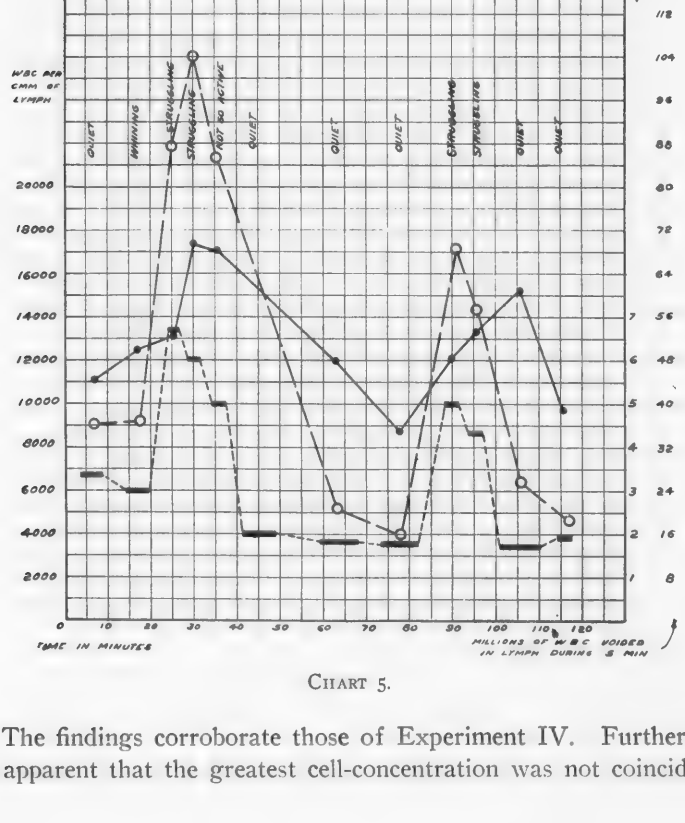

CHART 5.

The findings corroborate those of Experiment IV. Further, it is apparent that the greatest cell-concentration was not coincident

with the beginning of struggle, nor with the greatest lymph-flow, but came later. Indeed, that induced by the second struggle appeared after exertion had ceased. This second struggle did not bring out such a flow of lymph or mass of cells as did the first (which was similar in intensity); and, following both, the lymph-output, cell-concentration, and cell-output, all sank to below their level previous to the exertion.

In the next experiment the struggle was purposely made very long.

Experiment VI.—Spaniel; male; wt. 11 kilo; no food for 24 hours before operation. The duct was opened 10 minutes previous to the experiment proper, after it had undergone 5 minutes ligation. With whiffs of chloroform and tweakings of the skin a continuous struggle lasting 35 minutes was maintained. The lymph was slightly chyliform throughout; no clotting in tubes or cannula. Cell-counts were made in the order of tube-collection and between 2½ and 4 hours after this. (See Chart 6.)

At autopsy several tape-worms were found in the intestines.

This chart, while in general like the others, shows that the increase in cell-output during struggle is neither transient nor small. It endured so that at the end of the 35 minutes exertion there were being emptied from the thoracic duct 1½ times as many cells in each 5 minutes as during the preceding quiet. In the 35 minutes of muscular activity 48 cubic centimeters of lymph, containing an average of 5,100 white cells per cubic millimeter, were voided, as compared with 21 cubic centimeters of lymph, containing 3,100 white cells per cubic millimeter, in the 35 minutes just previous, or slightly over twice as much lymph, and, in sum, nearly four times as many cells as when the animal was quiet. Immediately following struggle there was a great fall in the total cell-output, and during the next 50 minutes it held to a low level.

In this series of observations the effects of five struggles were noted, and, during the work on lymphagogue action (*q. v.*), the effects of three more. They agree in these results:

(*a*) Struggle causes the cell-concentration of the lymph to become much greater. An attempt was made to test the parallelism of this increase with that observed in the lymph-flow, by collecting specimens at short intervals of time. This was done in Experiments V, VI, and during the struggle in Experiment VIII. The

charts of these prove that the maximum cell-concentration appears after considerable struggle-lymph has been voided, and at a time when the rapidity of lymph-flow is lessening. In one instance it was present in the slowly flowing lymph obtained on the return of quiet. These facts bear on the problem of whether the

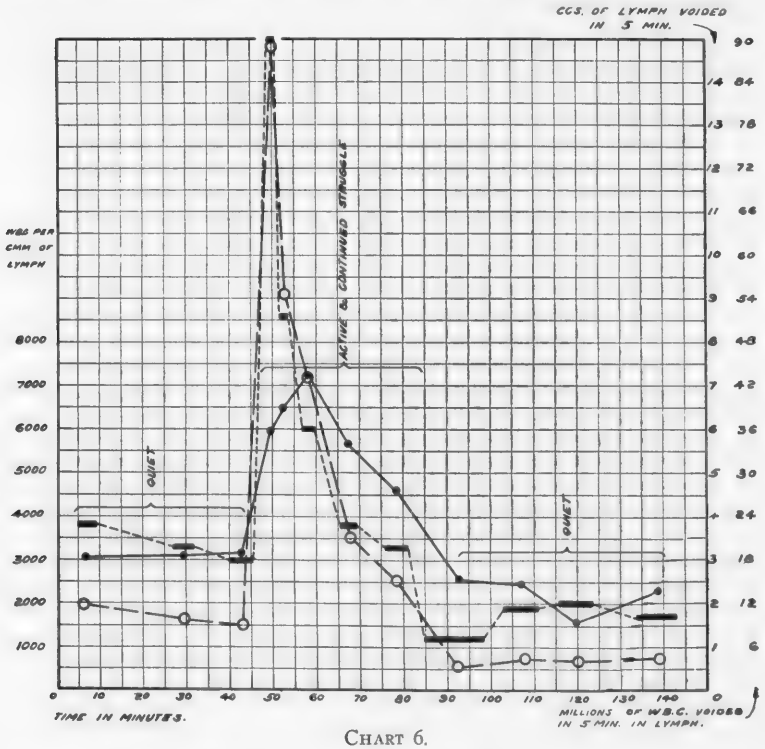

CHART 6.

cell-increase is due wholly to the flushing out of cells from the receptaculum chyli. Since the receptaculum is close to the opening of the thoracic duct, the cells flushed from it would appear, owing to the small size of the reservoir,[4] in the first few cubic centimeters

[4] I have made notes on the size of the receptaculum in several freshly killed dogs. The size varies widely, as does the shape of the reservoir, which indeed may not be present as such (Jussifow (25), and others). The walls of the receptaculum normally are held nearly apposed, so that the content is slight; but an impediment in the thoracic duct causes almost immediate dilatation with the accumulation of 1 to 4 c.c. of fluid.

voided during struggle; and, following this evacuation, the cell-increase would disappear quickly. But, in reality, the maximum cell-output was not infrequently delayed in its arrival (Experiment IV, second struggle; Experiment V, first struggle), and the maximum cell-concentration practically always was. In addition, the cell-increase is not transient (see Experiment VI) as would be the case were it traceable wholly to elements sedimented in the receptaculum.

Even granted that the first addition in cells comes from the receptaculum, whence is derived the later addition? Recent evidence (MacCallum (23), Buxton and Torrey (24)) speaks against v. Recklinghausen's view that the peritoneal cavity opens by direct channels into the lymphatics; and the quantity of free cells of this cavity is, normally, very small. The bulk of the increase is doubtless derived from the further lymphatic ramifications, in particular from the lymph-glands and other centers of lymph-cell formation. That the increased cell-concentration may persist for a short while after quiet has been restored is not surprising, since a host of cells, started from peripheral regions on the journey to the thoracic duct, need not arrive there until several minutes after the cause that got them under way had ceased to act.

(*b*) Struggle causes the cell-output by way of the lymph to become much greater. This effect persists throughout a struggle of considerable length.

In explanation of this phenomenon of increased cell-output it must be remembered that the lymph-region drained by the thoracic duct during struggle is larger than that during quiet. During the latter state, according to Starling (26), the fluid that arrives in the duct is derived, practically in toto, from the abdominal viscera. But muscular movement immediately forces from the limbs much lymph (Lassar, Cohnheim, Winternitz), as well as from the viscera; and, following this effect of direct pressure, there is a secondary increase in the flow from both sources, due to new lymph-production (Heidenhain, Starling). The territory of cell-supply opened for the first time, so to speak, by struggle, helps account for the greatly swollen cell-output.

(*c*) Following struggle, the cell-concentration and cell-output become for a time less than they would have been in the absence of muscular exertion.

This is demonstrated in the charts of Experiments IV, V and VI. The lessening in cell-output might be deemed merely such as was seen in the control animals of the first three experiments, were it not that the cell-concentration and the lymph-output (of which two the cell-output is the product) are both lower for a time than they would have been in the maintenance of quiet. One may suppose the glands to have been deprived of the majority of immediately available cells, and the slowed lymph-stream insufficient to wash with it all of those actually present.

These findings are, further on, dealt with in their clinical bearing.

Experiments in which the rapidity of the lymph's flow is varied without movement by the animal should provide increased light on the mechanism responsible for the results just described. The lymphagogue action of glucose was accordingly turned to this purpose.

The period of observation in these experiments was so short that it could not matter if the glucose acted to stimulate or retard cell-development. Other factors, though, demanded consideration. A possible effect of a lymph of high sugar-content to loosen elements from the glands, through changes in osmotic relations, could not be ruled out. Further, the lymph of struggle and of "glucosæmia" are not derived in similar proportions from the same regions.

There are three great areas of lymph-supply (Starling): The liver; the other abdominal viscera, in special the intestines, whence the lymph of the whole region may be designated "intestinal"; and the remaining portions of the body, the lymph from which may be termed "extremity-lymph." As has been said, the lymph of struggle comes from all of these sources, and in no small part from the limbs. The intravenous injection of glucose gives also increased lymph-flow from all the sources (Starling). The results of the procedures might, then, be directly compared, were the tissue forming lymph-cells equally distributed. But the liver of the dog possesses none of this tissue except that in the glands at its hilus (Ellenberger (27)), whereas the intestines and mesentery are

quite rich in it. The other body-parts possess, in proportion to their bulk, a very moderate quantity. Thus, of "mixed lymphs" from the thoracic duct, those derived most largely from the liver should be poorest in cells. So one must ask whether the lymphs of struggle and of "glucosæmia" are exactly similar in their derivation. To this only an approximate answer can be given. Starling has found that of the lymph obtained after the injection of glucose, much comes from the liver, less from the intestines, and relatively little from the other portions of the body. According to him increased blood-pressure and differences in permeability of the capillaries are responsible for the whole phenomenon. On the other hand the abrupt, initial increase in lymph-output induced by struggle is largely dependent on lymph previously present in the limbs, and now forced from them by the movements. Nothing analogous to this is caused by the glucose. The persistence of the large lymph-flow during struggle is traceable, however, to the same cause[5] as that following glucose injection, viz., increase in blood-pressure; and the resultant lymph is derived in much the same relative proportion from the three regions of production. Thus a rational basis is given to a comparison of the effects of glucose on cell-content with those observed in struggle after the initial increase in lymph-flow has subsided.

Experiment VII.—Mongrel; female; wt. 11.3 kilo; no food for 48 hours previous to experiment. The duct was ligated, and opened, 6 minutes before the beginning of lymph-collection. Lymph tinged with yellow, clear; no clotting in tubes or cannula. After three specimens had been got, 45 grammes of glucose in 72 c.c. of distilled water were injected slowly into the left subclavian vein. The animal remained absolutely quiet. The cell-counts were made in the order in which the specimens were taken, and 2½ to 3½ hours after their collection. (See Chart 7.)

At autopsy the animal proved to have been healthy; some round-worms were found in the intestines.

The results on cell-content are identical, as the chart shows, with those of struggle.

Experiment VIII.—Mongrel; female; wt. 16 kilo; no food for 48 hours previous to experiment. The duct was ligated during 10 minutes, and some stasis thus induced. On account of this, it was deemed safest to let the lymph

[5] This assertion might justly be objected to by those who oppose the theory of the mechanical formation of lymph. It stands or falls with that theory.

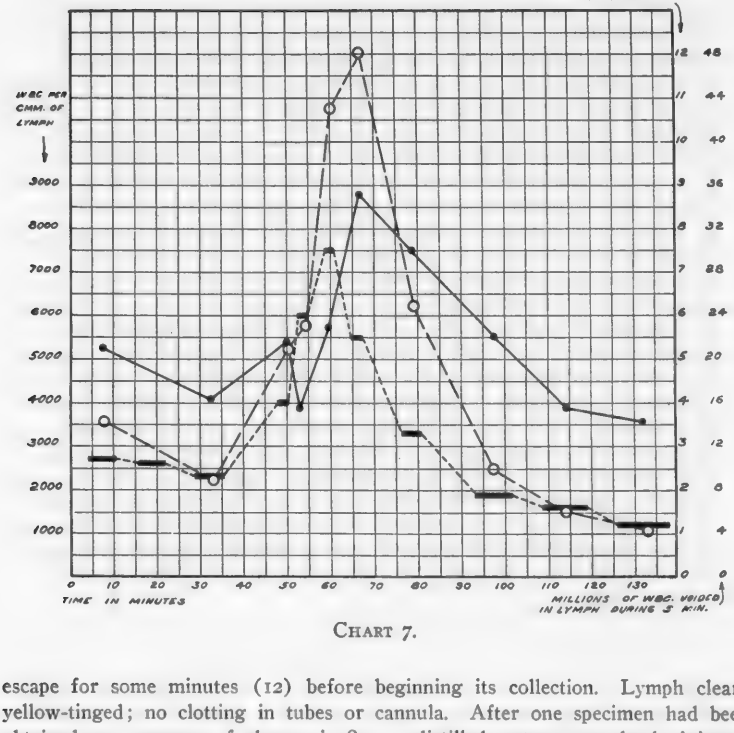

CHART 7.

escape for some minutes (12) before beginning its collection. Lymph clear; yellow-tinged; no clotting in tubes or cannula. After one specimen had been obtained, 52 grammes of glucose in 80 c.c. distilled water were slowly injected into the left subclavian vein. When the increase in lymph-flow due to this had subsided, the animal was made to struggle. Specimens were taken at frequent intervals. No chloroform was necessary, following its preliminary use to make anæsthesia complete. Cell-counts were made from the tubes in their order of collection and 2½ to 4 hours after that. (See Chart 8.)

At autopsy the animal was found to have been pregnant. There were ten embryos of an average length of 1 centimeter.

In this instance the effect of the glucose differed from that in Experiment VII. Here the cell-concentration rose, as result of the increased lymph-flow, whereas there it fell. In both cases the total cell-output became larger. In Experiment VII the curves were in all ways typical of those of struggle, whereas in Experiment VIII they were quite different, a fact which struggle in the same animal

helped to bring out. This struggle, coming after the glucose had operated, and causing an increase in the lymph but little superior to that from the glucose, was attended nevertheless by a cell-output vastly greater, and by the usual high cell-concentration.

These dissimilar results of glucose were puzzling. But the condition of the animal in Experiment VII had not been quite the

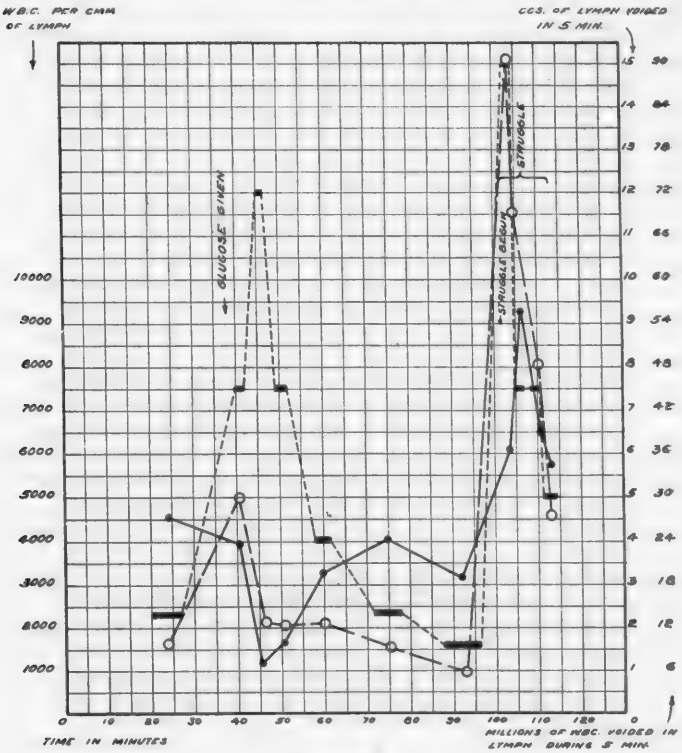

CHART 8.

same as that of the others. Owing to difficulty in the isolation of the thoracic duct, it had lain quiet under the anæsthetic 4 hours before the experiment proper began, instead of, like the others, only 1 to 2 hours. Perhaps, during this long period of preliminary quiet, the cells matured in the lymph-glands had in large part failed to be

carried away by the slow lymph-current, whence the marked appearance of them in the rush of fluid due to the glucose. On such reasoning it was determined to repeat the experiments, avoiding a long period of preliminary quiet, or flushing transiently the lymph-channels previous to the glucose injection by inducing restlessness in the animal.

Experiment IX.—Bull-dog; male; wt. 15 kilo; no food for 48 hours prior to experiment. The duct was ligated, and opened, 16 minutes before lymph-collection. The lymph was chyliform; no clotting in cannula or tubes. After one specimen had been obtained with the animal quiet, restlessness was brought

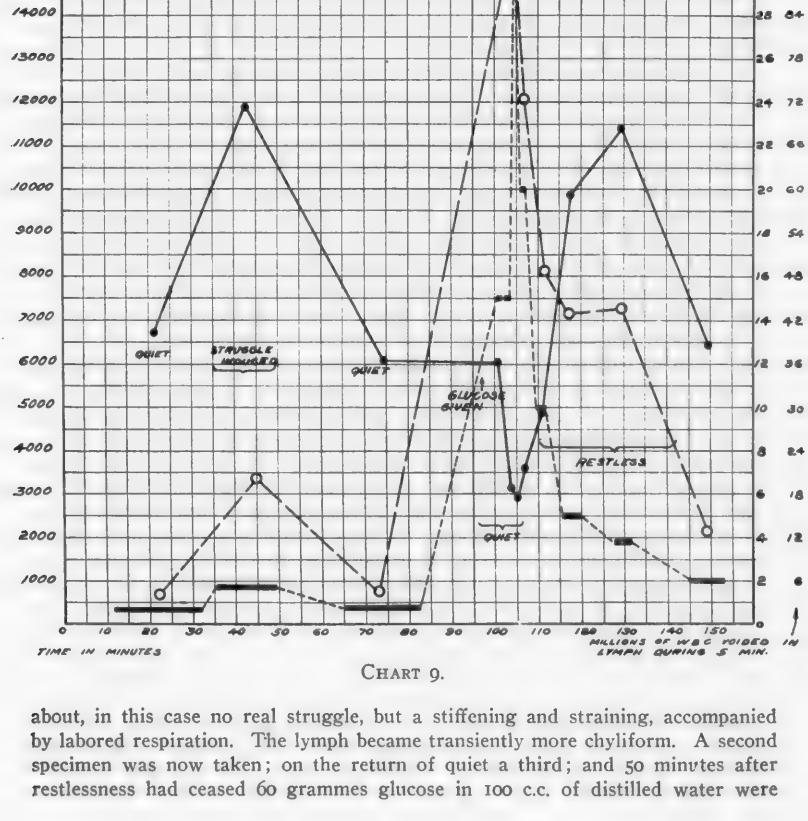

CHART 9.

about, in this case no real struggle, but a stiffening and straining, accompanied by labored respiration. The lymph became transiently more chyliform. A second specimen was now taken; on the return of quiet a third; and 50 minutes after restlessness had ceased 60 grammes glucose in 100 c.c. of distilled water were

injected slowly into the left external jugular vein. Before the lymphagogue action had disappeared the animal again became restless. Cell-counts were made in the order in which the specimens were taken, and 2½ to 3 hours thereafter.

The dog at autopsy proved to have been sound. Several large pieces of bone in the stomach accounted for the chyliform lymph. A tape-worm was found in the intestine.

From the chart it will be seen that struggle brought its characteristic effects on the lymph's cell-content, despite an extremely small increase in rapidity of flow. With the enormous lymph-output caused by the glucose, the concentration of the separate cubic millimeter of fluid was diminished, yet the cell-output as a whole became profoundly more.

Experiment X.—Mongrel; male; wt. 24 kilo; no food for 24 hours before experiment. The duct was opened 18 minutes previous to the beginning of lymph-collection. It had been ligated 8 minutes. After observations during quiet the animal was made to struggle during 2½ minutes, and 30 minutes later 100 grammes glucose in 100 c.c. of distilled water were injected into the left sub-subclavian vein. The lymph, which had been slightly opalescent, now became distinctly milky. There was no clotting in tubes or cannula. Except for the one struggle, the dog was quiet throughout. Counts of the tubes were made in the order of their collection and 1½ to 3 hours following that. (See Chart 10.)

At autopsy one tape-worm was found in the intestines.

No count was made of the lymph taken during struggle. The curves representing glucose action are similar to those of Experiments VIII and IX. In this instance such a rush of lymph was observed, and such a vast increase in total cell-output (despite lessened cell-concentration), as showed itself in no previous experiment. The curves representing these are much flattened.

The results justify the supposition that the high cell-concentration seen in Experiment VII is traceable to accumulation of cells during the long, preliminary quiet. Experiment VII shows that under certain conditions increased lymph-flow[6] may give results exactly similar to those of struggle.

To summarize the results with glucose:

(*a*) The increase in lymph-flow produced by the intravenous injection of a solution of glucose is accompanied by an alteration in the cell-concentration of the lymph. Usually the cell-concentration

[6] The change in osmotic relations caused by the glucose cannot be ruled out as a possible factor.

is decreased, but there is some evidence to show that, when the conditions have been such as to lead to the accumulation of cells in the lymph-system, it may be increased.

(*b*) With the increased output of lymph there goes an increase in total cell-output. This increase may be enormous. In Experiment X 5½ times as much lymph and 3½ times as many cells, were

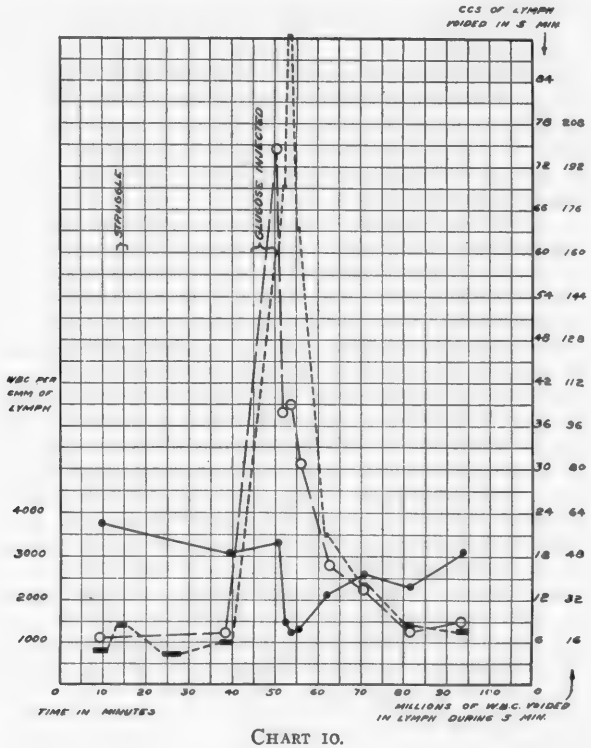

CHART 10.

voided in the half-hour immediately following the glucose administration as in the previous half-hour of quiet.

One may ask whether the results are not produced in the same way as those of struggle, and if they are not similar to them, except that the cell-concentration is rendered small by the distribution of the cells through a large amount of lymph. To put the question

directly, are the results of struggle those of simple increase in lymph-flow?

The evidence at hand is against this view. One may obtain a greatly increased cell-concentration during muscular activity in which the lymph-flow is quickened but little. Furthermore, increased lymph-flow by itself often diminishes the cell-concentration of the lymph, whereas struggle invariably heightens it. A direct comparison of the two procedures, such as Experiment VIII fortunately gave, demonstrates a difference in their results. In this experiment during the 11 minutes of muscular activity 19.25 cubic centimeters of lymph, with an average cell-content of 6877 cells per cubic millimeter, were voided; whereas, in the 11 minutes of greatest lymphagogue action, the nearly identical quantity of lymph voided (19.8 cubic centimeters) held only 2,267 cells per cubic millimeter. Differences in amount of lymph-flow alone could not be responsible for these variations of the cell-concentration in opposite directions and the differences in total cell-output.

It is true that, as already discussed, the lymphs obtained by the two methods may not be derived entirely from the same regions. The first "struggle lymph" is made up largely of that already present in the extremities, whereas that of the glucose is "liver-," "intestine-," and "extremity-lymph," in much the same relative proportions as that produced late in struggle.

It is questionable whether the cell-content of the "extremity-lymph" forced into circulation at the beginning of struggle could increase the cell-concentration of the "mixed lymph" of the thoracic duct, since it comes from a territory poor in lymphadenoid tissue, and has, indeed, been shown to hold normally fewer cells per cubic millimeter than does this "mixed lymph" (Pohl, Winternitz). But leaving this to one side, we are permitted a comparison of the effects of glucose and those that appear in struggle after the first rush of lymph has ceased. In the one instance the cell-concentration is diminished, in the other it is heightened.

One must conclude that another factor besides increase in lymph-flow per se helps during muscular activity to increase the cell-output. This is not hard to imagine. Direct pressure effects on the lymph-stream to set in motion those cells that had settled in the vessels,

and to scour the glands of mature elements, should certainly play a part. The work of Harvey (29) on the lymphocytosis caused by pilocarpine indicates that it is brought about by contraction of the smooth muscle in the capsules of the lymph-glands and spleen. Thus another possible action of struggle is suggested. Whatever the factor or factors may be, they are probably quite as influential during struggle to produce the large cell-output as is the accompanying increase in lymph-flow, which Ehrlich holds to be alone responsible for all heightened output of lymph-cells not dependent on their more abundant maturation. Nevertheless the theory that increase in lymph-flow gives increase in cell-output, is supported by the results of the lymphagogue action of glucose. The only objection to these as direct proofs of it is that an effect from the changes in osmotic relations brought about by a lymph of high glucose content cannot be ruled out.

It should be noted, in observing the charts as a whole, that the variations in cell-output are in keeping, quantitatively, with the amount of cell-output during quiet, the " cell-capital," so to speak. Succeeding to variations the cell-output tends to return close to its height previous to them. The fact that it usually becomes somewhat less than before them,—an indication of that gradual diminution in it observed in animals from which the lymph is gradually drained (Experiments I, II and III), or, in some cases (Experiments V and VI) of partial exhaustion of the supply of mature cells, —does not affect the principle. We may say that the cell-output seems " set " to maintain a stable rate during some hours at least. Healthy, adult dogs, kept, so far as possible, under the same conditions, differ widely in this rate of cell-output: in Dog Gl more than nine times as many lymph-cells per kilo of body-weight are furnished to the blood through the thoracic duct as in Dog Fl. Does this mean a difference in amount of cell-production by the tissues? The appended table helps answer this question.

From this table it is clear that, while the cell-output per kilo of body-weight does not depend on size of the individual, or on differences in length of fast,[7] it has perhaps a relation to rate of lymph-

[7] Firleiewitsch (30) has found the lymph-glands of well-fed rats to be more numerous and larger than those of starved ones; but he assigns this to a larger size of the cells making up the tissue, not to a greater number of them.

Dog.	Length of Fast.	Weight in Kilos.	Average No. of w. b. c. per cmm. Lymph.	Average Flow of Lymph in 5 Minutes.	Total w. b. c. in this Amount of Lymph.	Total w. b. c. in Amount of Lymph Furnished per K. of Body-weight in 5 Minutes.	Flow of Lymph per K. of Body-weight, in 5 Minutes.
Ll, bull, male	48	15	6,700	0.7	4,690,000	312,666	0.05
Hl, terrier, male	24	11	4,960	0.75	3,720,000	338,181	0.07
Pl, collie, male	48	13	2,900	1.5	4,350,000	334,615	0.11
Al, bull, female	24	15	3,600	2.0	7,200,000	480,000	0.13
Kl, collie, male	48	16	4,510	2.3	10,373,000	648,313	0.14
Gl, bull, male	24	18	11,160	3.3	36,828,000	2,046,000	0.18
El, setter, male	24	20	8,400	3.75	31,500,000	1,575,000	0.19
Ml, setter, male	24	24	3,780	4.6	17,388,000	724,500	0.19
Ol, collie, male	48	23	6,760	4.6	31,096,000	1,352,000	0.20
Cl, mongrel, male	2	10	1,500	2.0	3,000,000	300,000	0.20
Nl, terrier, female	48	8	4,180	1.7	7,106,000	888,250	0.21
Jl, mongrel, male	48	11.3	4,640	2.5	11,600,000	1,026,540	0.22
Fl, mongrel, male	24	19	990	4.3	4,257,000	224,053	0.23
Il, spaniel, male	24	11	3,000	3.8	11,400,000	1,036,363	0.34

flow per kilo of body-weight, being, on the whole, least in those individuals in which this flow of lymph is least. It may be supposed, in the light of what has gone before, that the lymph-flow, as an agent of transportation, is here responsible for the differences in cell-output, rather than by stimulation of the cell-forming tissues; in special since, so far as we know, this rate of cell-output is constant only from hour to hour, and may not be so from day to day. Some of the wide differences may be due to the presence of an accessory thoracic duct, which is not infrequent in the dog (Biedl and v. Decastello), or to other channels conveying a share of the elements that would, normally, course through the thoracic duct. In association with the idea of actual variation in the productive activity of the cell-forming tissues, may be cited the work on ruminants of Forgeot, already quoted. He found the cell-output of young individuals to be markedly greater than that of adults. Differences in age of the animals may be at the root of some of the differences in cell-output. But the mechanical factors, just cited, will explain the larger differences, and make needless a further entrance into the dark subject of activity in the cell-forming tissues.

The cell-concentration of the lymph of the dog, as determined under the conditions of operation outlined at the beginning of this paper, has a worth in its relation to the cell-content of the blood.

Delamere, Winternitz, Goodall and Paton, Dastre, Henri and Stodel, Beidl and v. Decastello, and Ranvier give figures ranging from 1,372 to 22,729 white cells per cubic millimeter. The results in the table, as might have been expected from the technic employed, do not exhibit such wide variation. From them one may judge this "normal" cell-concentration of the "mixed lymph" of the dog to lie between 990 and 11,160 cells per cubic millimeter, with an average of 4,000 cells.

The most important outcome of this work is the discovery that the system forming the lymphocyte possesses large reserve power to increase transiently its output of cells. During muscular activity this may, for the space of a half-hour, be nearly four times what it is in quiet, as has been shown. A clinical application of the finding is not far to seek.

For this application it is necessary to know what white cells the lymph furnishes the blood. These are in the dog much the same as in man, just as the leukocytes of the dog's blood resemble in general those of man both in morphology and in relative proportion of number (Dawson (31), Tallquist and Willebrand (32), Busch and Van Bergen (33)). The bulk of cells furnished through the thoracic duct of the dog is one of lymphocytes, large and small, but a few large mononuclears, a varying, small percentage of eosinophiles, and an occasional polymorphonuclear neutrophile are also present (Delamere, Biedl and v. Decastello). The lymphocytes alone exist in sufficient quantity to be important to the blood.

Since, as has been emphasized, the thoracic duct furnishes a large, if not the greater part, of the blood-lymphocytes, an increase in the lymph's cell-concentration should produce, other factors being equal, an absolute lymphocytosis in the blood. When the amount of lymph is at the same time increased, thus multiplying the cell-output, the effect should be more profound. Thus one would expect struggle to produce, clinically, an absolute lymphocytosis.[8]

[8] But it must be assumed that the change in cell-output is not accompanied by change in cell-formula. I have made repeated counts of the lymph before and during struggle, and have found no such change.

The clinical records of blood-counts following muscular exertion indeed show the presence of an absolute lymphocytosis. Schultz (34), and later Winternitz, observed a leukocytosis following exertion, but they did not note its kind. Burrows (35) found in the normal individual, after short exercise, a distinct increase in number of all the white cells, but especially of the lymphocytes. Capps (36), previously, had studied the blood during the convulsions and apoplectic attacks of general paresis, and, here too, had observed leukocytosis, most marked in the large mononuclear elements, but affecting the lymphocytes. Burrows went further in making plain the fact that the leukocytosis associated with true convulsions in the course of paresis is invariably of the inflammatory type, the polymorphonuclear neutrophiles giving the increase. He concluded, from his findings made during muscular exertion, that in convulsions two leukocytoses are really involved, a transient "physiological," wherein occurs an increase of all the elements, most marked, as his records show, in the lymphocytes, and a more pronounced and enduring "pathological" one.

Violent and long-continued physical exertion will itself produce a profound leukocytosis. Thus Larrabee (37) observed it as an effect of a 25-mile foot-race; and he decided that, here too, a "pathological" is superimposed upon a "physiological" leukocytosis, "an increase in cells all along the line," as he puts it. Only this "physiological" leukocytosis is of interest here. Larrabee, Tileston and Emerson (38), studying the blood after a similar race, confirmed these results. Further, the lymphocytes at the end of such exertion form a very small percentage of the total, having retreated to their absolute number during quiet, or even below it. We do not know how far destruction of the lymphocytes in circulation effects this and how far it is brought about by a lessening in the supply of them. The experiments reported in this paper show that a diminution in the cell-output by way of the lymph follows prolonged struggle.

Coming to more debatable ground, the well-defined lymphocytosis that accompanies whooping-cough may be referred to. This cannot be primarily due to the mechanical effects of struggle, since it appears early in the disease; yet the fact that it is at its greatest during the period of violent coughing (Meunier (39)), is suggestive. Un-

fortunately there are no published figures dealing with the leukocyte count immediately before and after a coughing-fit.

The experiments with a lymphagogue throw a little light on the vexed subject of digestive leukocytosis, particularly on the reason that it is vexed. The frequency of this leukocytosis, and the part played in it by the mononuclear element, varies with nearly every investigator who has applied himself to the problem. The discordance in results is, to a certain extent, explicable in terms of the conditions dealt with here. Quiet previous to a meal would predispose, on increase of the lymph flow during digestion, to a cell-output such as that in Experiment VII, which would probably swell quite markedly the number of lymphocytes in the blood. Any exertion after the meal would tend to make this output greater. Exercise previous to a meal, by flushing out the reserve of mature cells, would act to prevent an increase in cell-output during digestion, and this result would be made the surer by slow lymph-flow, were but little fluid ingested with the meal. Under such circumstances the blood-content in lymphocytes would not increase.

I am aware that, considering the many factors which must enter into the determination of the blood's content in lymphocytes, this discussion is one-sided. Yet, whether the hypotheses presented above be sound or not, the work on which they are based indicates one direction in which it may be possible to simplify some of the problems connected with the leukocyte.

CONCLUSIONS.

1. The lymph of the thoracic duct furnishes to the blood a larger proportion than is usually supposed of the lymphocytes in circulation. Gross variations in its output of such cells must affect very considerably the blood picture.

2. The quantity of lymphocytes supplied through the thoracic duct of the healthy dog remains practically constant from hour to hour, if the physiological conditions are not notably changed. Transient change in physiological conditions may alter the output of cells, but with the disappearance of this change the output tends to resume its previous rate. These facts indicate that the tissues producing lymphocytes are "set" at a rate of activity definite in the individual.

3. Muscular activity (struggle) produces a prompt increase in the output of lymphocytes through the thoracic duct.

(*a*) This is assured by the presence of an increased number of cells per cubic millimeter of lymph, combined with an increase in the amount of lymph voided.

(*b*) The lymphocyte-output may be tripled or quadrupled during a long-continued struggle.

(*c*) Following prolonged struggle the output of lymphocytes is for a short time less than previous to the exertion.

4. The increased lymph-flow caused by a lymphagogue of the second class (glucose) brings with it increased output of lymphocytes through the thoracic duct.

(*a*) The individual cubic millimeters of lymph are often poor in cells, during the rapid lymph-flow, yet the total number of elements transported is large.

(*b*) The results with glucose support the theory of Ehrlich, that a rapidly appearing lymphocytosis may be produced through the flushing effect of increased lymph-flow.

5. A comparison of the effects of struggle with those of glucose demonstrates that in the former some factor besides increase in lymph-flow per se (Ehrlich) works to cause the large output of lymphocytes. The nature of this factor has not yet been determined.

6. The variations caused by muscular exertion and by increased lymph-flow in the number of lymphocytes coursing through the thoracic duct are so pronounced as to suggest that the total number of lymphocytes in circulation must be considerably influenced by them. Clinical findings by other observers indicate that this is true; and the clinical findings themselves become much simpler of interpretation.

7. The results in general prove the existence, reserved from circulation, of a large fund of lymphocytes, which is quickly yielded to the blood under certain physiological conditions.

I wish to thank Dr. Warthin for an interest in the work that has been most helpful.

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CONCERNING THE RELATION OF THE COAGULATION TIME OF THE BLOOD TO THROMBOSIS IN PHLEBITIS.[1]

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The etiological factors or conditions concerned in the production of thrombosis may be briefly summarized as:

1. Central or peripheral slowing of the blood stream.
2. Lesions in the walls of the blood vessels.
3. Alterations in the blood itself, such as tend to favor coagulation.

In actual cases, it appears almost without exception that two or more of these factors are associated in the production of thrombosis.

Although the etiological agents mentioned above are generally accepted as correctly explaining thrombosis, it must be recognized that experimentally as well as clinically very uncertain results follow when we so attempt to explain concrete instances, notably such as occur in clinical phlebitis. Until more certain data are secured in regard to this process, but little can be expected in the way of successful prophylactic treatment or in the certain determination of those instances in which this lesion is to be feared.

The object of this brief study has been an attempt to show what part artificially increased and decreased coagulability of the blood plays in the production of thrombosis, or, expressed in other words, whether in conditions productive of phlebitis, thrombosis is more likely to occur when the coagulation time of the blood has been lowered artificially or less apt to take place when analogous means have been employed to prolong the coagulation time of the blood.

Our experiments have been conducted on a series of young and

[1] Received for publication, January 2, 1908.

healthy rabbits, one third of which had their coagulation time reduced by daily administration of 2 grams of calcium lactate; an equal number of animals in which the coagulation time had been artificially lengthened by daily dosage of 2 grams of citric acid; the remaining third was used as control animals. The drugs were introduced into the stomach by tube feeding and it was found possible, with this dosage, to reduce the coagulation time of the blood in the calcium lactate animals one half and to lengthen it in the citric acid animals about one third. As corroborative of work already well authenticated, we found that the maximum effect of these drugs takes place about two hours after their administration and probably passes entirely away within twelve hours, especially in the case of the rapidly excreted calcium salts. In our animals the medication was continued in daily doses throughout the experiment, since it was found that effects were quite as likely to occur several days after the initial injury as immediately on its infliction, thus also more closely approximating the conditions occurring in man.

When autopsies were to be performed the animals were chloroformed and while the heart action was still vigorous a carotid artery was opened and the animal suspended so that the blood was very generally emptied from all the vessels of the body, and post-mortem clot or fibrin could not become confused with true thrombi. We believe this to be an important technical step.

Our experiments may be grouped in two series:

Series I comprises local injuries produced in and around the ear veins. Nine experiments of this character were performed.

Series II includes attempts to produce vascular lesions predisposing to phlebitis and thrombosis by intravenous injections of various irritants. Five sets of experiments were made.

Series I. *Experiment a.* A 3 centimeter segment of the distended marginal ear vein was isolated by compression between two artery clamps and the intervening vein distended with blood was compressed and lacerated with toothed forceps for five minutes when minute hæmorrhagic extravasations along the course of the trunk were demonstrable. The isolating clamps were then removed and the circulation allowed to become reestablished. The resulting peri-venous inflammation was slight and no thrombosis followed either in the calcium, citric acid or control animal.

Experiment b.—Clamping the marginal vein with ordinary paper clips, cutting off at the same time the anastomosing circulation for thirty minutes, was followed by immediate reestablishment of the normal blood flow in all animals without subsequent results.

Experiment c.—A 2 centimeter segment of the marginal vein was clamped and isolated with paper clips for twelve hours, the anastomosing circulation being meanwhile prevented. Immediately on the removal of the clips the circulation was reestablished in all animals. The absence of thrombosis in these experiments was similar to the results of the experiments reported by v. Baumgarten (1) and his student, Rizor (2), who was able to compress the vein for an even greater length of time without resulting thrombosis. After three days considerable inflammatory reaction developed about the site of some of the clamps, being most marked in the calcium lactate animal, where finally a small segment of the vein became thrombosed. The inflammatory reaction was not so marked in the control animal and there was still less reaction in the citric acid rabbit, thrombosis being absent in both.

From these experiments one may conclude that mere stagnation of the venous blood produced no marked tendency toward thrombosis in the ear veins but that inflammatory lesions, with consequent phlebitis are more extensive in the case of the calcium animal while thrombosis occurred only at the immediate point of injury of the vessel walls.

Experiment d.—A quantity of 24-hour growth of virulent pneumococci in bouillon was injected about the ear vein, the injection being continued to such a point as to cause compression anæmia of the desired segment of the vein. The circulation was shortly reestablished and the amount of subsequent inflammatory reaction was slight. No thrombosis occurred in any of the animals. Our results in this experiment are therefore quite unlike those of Talke (3), where, however, the coagulation of the blood was not altered, for Talke claims to have regularly produced thrombosis in this way.

Experiment e.—Five minims of 5 per cent. silver nitrate solution were injected into the peri-venous connective tissue of the ear. Immediate permanent thrombosis of the adjacent vessels followed. No difference existed in extent or degree between the three animals. This experiment was repeated using 1 per cent. solution of silver nitrate. Slight peri-vascular inflammation without thrombosis resulted and was of about equal severity in all the animals.

Experiment f.—Three drops of pure turpentine were injected between the branches of the median ear vein. This was followed within twelve hours by œdema and an active inflammatory exudation with thrombosis of the involved vein in all three animals. The thrombosis was notably more extensive and resolution most delayed in the animal which had received the calcium lactate, less so in the control and least of all in the rabbit which had been poisoned with citric acid.

The same relations, as regards severity and occurrence of lesions, followed when 50 per cent. turpentine in an inert oil was

employed in the experiment except that the resulting inflammation and subsequent thrombosis was longer delayed.

SERIES II.—Finely comminuted sterile pumice was injected into the marginal ear vein in an attempt to see if the resulting thrombosis, following probable embolism of the terminal arterioles, would be more extensive in the animal that had received the calcium salt and less in the one that had received citric acid. All three animals recovered perfectly from the operation and later autopsies showed no lesions whatsoever which could be attributed to this injection.

Experiment b.—Fifteen minims of sterile cod liver oil were injected into the marginal ear veins of the three animals. None of them showed symptoms and later post mortems showed no lesions attributable to the experiment.

Experiment c.—Ten minims of a pure 24-hour culture of virulent typhoid bacilli were introduced through the marginal vein. No symptoms of illness followed and these animals were subsequently utilized in experiment *d*. These results are exactly contrary to those of Jakowski (4) who in similar experiments on guinea-pigs and rabbits obtained almost constant thrombosis, without the associated employment of calcium or citric acid.

Experiment d.—Fifteen minims of a suspension of a 36-hour broth culture of virulent typhoid bacilli in an equal bulk of sterile cod liver oil were introduced through the marginal ear vein. This was done in the expectation that embolism caused by the oil would be likely to afford sites for the growth of the bacilli thus leading to thrombosis as described in the experiments of Jakowski (4). All the animals became seriously sick and died, on postmortem examination multiple serous petechiæ, general parenchymatous degeneration, lymphadenitis and frequent infarctions were found, but no general or isolated thrombosis. The lesions were notably most extensive in the calcium lactate animal and least extensive in the citric acid, but when the experiment was repeated under similar conditions, exactly opposite results followed, which leads us to believe that chance was really the controlling factor in determining these changes.

CONCLUSION.

Positive results have been obtained in but a single set of experiments, namely those in which turpentine was employed.

In so far as the results of this preliminary study go, one is led to the conclusion that thrombosis is most readily induced when active inflammatory lesions exist in the blood vessels, associated, probably in most instances, with secondary degenerative changes. Purely mechanical lesions are much less apt to be productive of conditions favorable to thrombosis as a sequence of phlebitis.

Marked artificial increase or decrease in the coagulation time of the blood by the use of calcium lactate or citric acid, does not render animals abnormally prone to thrombosis incited by changes other than inflammatory.

When true phlebitis exists, thrombosis is apt to be more extensive and less readily resolved, when the coagulation point of the blood has been shortened by the use of calcium lactate, and it is less extensive and more quickly absorbed when the coagulation time has been increased by the administration of citric acid.

Experiments as yet incomplete appear to suggest that the increasing in rapidity or slowing of the general circulatory stream has but little bearing on the production of thrombosis in phlebitis, much less, indeed, than clinical and anatomical observations have generally led us to think. We have also been led to suspect that the presence or absence of anastomoses of abundant degree is largely concerned as a factor in determining the location and extent of thrombosis in phlebitis.

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CALCIFICATION OF THE ARTERIAL SYSTEM IN A CAT WITH TRANSPLANTED KIDNEYS.[1]

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In a previous paper,[2] the history of a cat,[3] whose kidneys were extirpated and replaced by the kidneys from another cat, and whose arteries underwent afterwards extensive calcification was incompletely reported. It was the first observation showing that a very slight morphological change of the kidney might be followed by intense and rapid arterial degeneration. On account of the importance of this fact it seemed advisable to summarize the clinical history of the animal and to describe fully the gross and microscopical findings of the autopsy.

The animal operated on was a young adult female cat in excellent health which had lived in the laboratory for several months. Her kidneys were resected, the abdominal aorta was dissected and found normal, and both kidneys from a middle aged female cat, which was also in good health and whose arteries were normal, were grafted into her abdominal cavity. The animal recovered quickly from the operation and her life went on just as before. Fifteen days after the operation, both kidneys were movable and normal in size. The animal urinated and lived as a normal cat. Seventeen days after the operation, both kidneys were found very much enlarged and fixed to the lumbar wall, and the urine contained a great deal of albumin. An exploratory laparotomy, performed on the eighteenth day, showed both kidneys very much increased in size. Their consistency was softer than normal. The lumbar peritoneum was incised on the middle line, and dissected. The arterial and venous circulations appeared to be normal. The connective tissue of the hilus was œdematous, and clear fluid flowed

[1] Received for publication, January 7, 1908.

[2] Alexis Carrel, *Jour. of Exper. Med.*, 1908, x, 98.

[3] Experiment 14.

from it. The wall of the ureter was œdematous also. The color of both kidneys was rosy and normal; there was no congestion. The peritoneum covering the anterior face of the right kidney was dissected and retracted and the capsule incised; clear fluid and red blood flowed from the incision. Then the renal tissue was incised and found œdematous, but not congested. An abundant hemorrhage of red blood followed, which was controlled by suture of the capsule with very fine silk. No suture of the lumbar peritoneum was made. The abdominal wound was closed as usual. After the operation, the quantity of albumin decreased rapidly. The size of the kidneys diminished progressively and was almost normal fifteen days afterwards. Nevertheless the animal became emaciated, and, without having presented any definite symptoms, died on the thirty sixth day after the transplantation.

The autopsy was performed one hour and a quarter after death.

Macroscopical examination.—On the abdominal wall, two transverse scars are observed. The lower one is linear and hardly discernable; it is the scar of the first laparotomy. The upper one, a little wider and very apparent, is the scar of the second laparotomy. To palpation, these scars present extremely different characteristics. The scar of the first laparotomy is a normal, narrow and elastic scar, while the scar of the second laparotomy is very wide, irregular, and its consistency is extremely hard, as if a rib had developed in the abdominal wall.

After incision of the wall, it is found that the scar of the first laparotomy is entirely normal, without any apparent infiltration of lime salts. On the contrary, the scar of the second laparotomy is so hard that it is difficult to cut it with the scissors. The wall is infiltrated with masses of lime salts, white in color and almost as compact in structure as a stone of the urinary bladder. Nevertheless, at the level of this scar, the peritoneum appears to be normal. It presents a white color, which is due merely to the deposit of lime salts in the subperitoneal connective tissue.

The abdominal cavity was opened by a large transversal incision. There are a few adhesions of the great omentum to the scar of the second laparotomy and no adhesions of the intestine. There is no fluid in the abdominal cavity and the parietal peritoneum is normal everywhere.

The small intestine was eviscerated. Its connections are normal. However, a loop of the first part of the jejunum is adherent to the scar of the lumbar peritoneal incision made during the second operation, and is sharply kinked at the level of the lower part of this incision. Nevertheless, there is no obstruction of the lumen of the intestine. The large intestine is normal. The omentum and mesentery are normal. The superior mesenteric artery is larger than normal; its consistency is very hard, and it can be broken by pressure as though it were a glass tube. It seems also that the consistency and size of the smaller mesenteric branches are markedly increased.

The stomach is apparently normal, but its arteries are dilated and very hard. The liver, the spleen and the pancreas are normal.

The kidneys are in their normal position and covered with normal peritoneum. The cat being emaciated, there is little adipose tissue around the kidneys, and the peritoneum is very transparent. The color of the organs is normal, and also their consistency. Their size is slightly enlarged, but within normal limits. They are movable on the lumbar wall, as normal kidneys are. The loop of jejunum which was adherent to the incision of the second lumbar peritoneal incision having been detached, it is found that the scar of this incision is markedly infiltrated with lime salts. By examining more carefully the peritoneum covering the right kidney, a white longitudinal spot is seen about two centimeters external to the hilus. This spot is hard in consistency and composed of a deposit of lime salts in the subperitoneal connective tissue, between the peritoneum which is sound and the capsula of the kidney which also is normal. This deposit of lime salts corresponds approximately to the place where the vertical subperitoneal exploratory incision of the right kidney was made. The healing of this incision has been so perfect that very little evidence of it can be found by a most careful examination in the capsule or in the renal substance. Its location is marked only by the infiltration of lime salts into the subperitoneal tissue, which has followed probably a very small hemorrhage from the line of suture of the capsule. The renal veins and arteries are normal in size, direction and consistency. The connective tissue surrounding them is normal also. The transplanted segments of aorta and vena cava are normal, and the anastomoses are excellent. But, the line of suture of the arterial anastomoses is as hard as a ring of wire, and there is a sharp difference at this point between the transplanted aorta which is elastic and soft, and the host's aorta which is as hard as glass.

The ureters are normal. A few centimeters below the kidneys, they become adherent to each other and enter the peritoneal cavity through the lower part of the lumbar peritoneal incision. After having passed along the right side of the rectum and above the uterus, they reach the bladder. They are in excellent condition and their small vessels appear to be normal. The transplanted flap of bladder is perfectly united to the bladder which is normal in appearance and in size. Its anterior face is longitudinally incised. About five cubic centimeters of yellow clear urine, containing albumin are found. The openings of the ureters are distinctly seen and are normal. The transplanted mucous membrane is normal and the scar between it and the mucous membrane of the host is almost undiscernable.

Then the kidneys were dissected and directly examined. The capsule is normal in appearance. At the level of the anterior face of the left kidney, there is a narrow brownish line which is probably the scar of the exploratory incision. The adhesion of the capsule to the kidneys is normal, and not increased at the level of the scar. There is no dilatation of the stellate veins. Both kidneys were opened. There is no dilatation of the pelvis. The relative dimensions of the cortex and medulla are normal. The medulla is normal. The cortex is normal but a little pale.

The suprarenal glands are normal.

In opening the thoracic cavity it is found that the cartilaginous part of the ribs is very hard and friable and intensely calcified. The internal mammary arteries are also calcified. The pleura, the trachea and the lungs are apparently normal. On a macroscopic section, the pulmonary tissue seems normal, but, on palpation its consistency is found to be greatly modified by the presence of a great many very small and hard foreign bodies. These were proved to be the calcified ends of the small bronchioles. The larger bronchi are normal, and their rings do not present any calcification. However, thick calcific nodules are found from distance to distance on the external layer of the larger bronchioles.

The heart is very small, much smaller than the heart of a normal cat of similar size. There is no apparent calcification of the coronary arteries. The valves are normal. Nevertheless, as the aorta is very much dilated, there was probably a marked degree of aortic insufficiency. The arch of the aorta, the brachio-cephalic and carotid arteries, and the descending aorta, have the consistency of glass tubing and are exceedingly friable. The arch of the aorta was incised longitudinally. It is very hard to cut with the scissors, especially near the heart just above the sigmoid valves. There is at this level a very large accumulation of lime salt. The dimensions of the vessel are very much increased. The internal circumference of the aorta near the heart is 26 millimeters, the same dimension in a normal cat being about 18 millimeters. The internal surface is of a yellowish-white color, with a reticular appearance, the elevated parts being more yellow and harder, while the depressed parts are whiter and a little softer. It corresponds to the differences in the intensity of the lime infiltration. But the wall, over the entire circumference of the vessel, is infiltrated and rigid. It is not a lesion localized in a few patches, but a diffuse one generalized over the whole vessel. However, near the mouth of the brachio-cephalic arteries there are thicker rings of lime infiltration. On the descending aorta, the infiltration is a little less marked and more regular.

The wall of the aorta is thinner than normal in several places. On the upper part of the arch, which is very much dilated, and, just in front of the mouth of the left brachio-cephalic artery, there is a part so thin that it would have probably broken under the aortic pressure had the animal lived a little longer.

The lesions of the pulmonary artery are very different. The dilatation is not marked and the wall has an almost normal consistency. But there are intense focalized calcific lesions. Just above the sigmoid valves, on the convex part of the vessel, there is a long calcified patch of yellowish color, seven millimeters in length, and raised above the intima to a height of more than one millimeter. Other patches are observed near the bifurcation of the pulmonary artery and in its branches. Between the patches the wall seems normal. The smaller branches of the pulmonary arteries are found enlarged and infiltrated with small white patches.

Diffuse calcification and thick circular rings are found on the wall of the carotid arteries. The subclavian and humeral arteries assume the same appearance. Even the small muscular branches of the humeral arteries are calcified.

The abdominal aorta presents diffuse lesions almost similar to those of the carotid and subclavian arteries, that is, diffuse calcification with thicker rings from distance to distance. These lesions stop sharply at the point of the anastomoses, and the transplanted aortic segment is soft and normal.

The calibre of the abdominal aorta is very much increased. The cœliac artery is calcified and dilated. The upper mesenteric artery is also completely calcified, and its lumen is as wide as this of a normal aorta.

The iliac and femoral arteries are affected with similar lesions.

Microscopical Examination.—The specimens were fixed in Zenker's fluid and in formalin and stained in hæmatoxylin and eosin. The arteries were cut without having been decalcified. Some of the arterial sections were also stained with Weigert elastic tissue stain. Dr. Simon Flexner had the kindness to look over the sections for me.

The Kidneys.—Beneath the renal capsule, in the superficial part of the cortex, there is a marked œdema which extends about one half millimeter downwards and then disappears. It is not regularly diffused but is more marked in some places than in others. There is also a focalized increase in connective tissue immediately beneath the capsule, chiefly about greatly dilated blood vessels, with heavy fibrous walls. The latter are dilated superficial veins. Surrounding them, there is a rich infiltration of small round cells. In one of these small veins, an organized thrombus projects into the lumen of, but does not occlude the vessel.

The secretory tubules are on the whole remarkably well preserved and there is no increase in connective tissue around them. On the other hand, groups of collecting tubules, high in the cortex, occasionally show an increase in the surrounding connective tissue, dilatation and either casts or cast material, which are composed in part of disintegrated blood corpuscles. Following the collecting tubules into the medulla, one finds focalized infiltrations around them of small round cells.

In the glomeruli, Bowman's capsule is not increased in thickness. For the most part, the capillary loops fill the capsule. The endothelium stains sharply and the capillaries contain blood corpuscles and no excess of leucocytes. The capillary walls are not thickened. There is no fluid in the capsular space.

No calcification is detected in any of the renal structures.

The liver, the spleen, and the intestines are normal. There is no change in their small vessels.

There is no lesion of the lungs. But the cartilaginous rings and plates of the middle sized and small bronchioles are intensely calcified. There is no calcification of the rings of the large bronchi and of the trachea. A section of the upper part of the cervical medulla is found normal and there is no lesion of its small vessels.

The suprarenal glands are entirely normal.

The Arteries.—The segment of aorta which was examined belonged to the middle part of the descending thoracic aorta. The intima appears normal. The muscular coat shows a very great reduction in the number of the nuclei ordinarily present. This reduction begins to be visible in the layer beneath the endothelium and becomes progressively more marked as we proceed outwards toward the adventitia. As the nuclei disappear, the muscular cells become more and more confluent and converted into a highly refractive material. As the adventitia is approached, lime salts begin to be deposited. The external musculo-elastic layers of the media, and the adventitia are extensively infiltrated with lime salts. There is a less degree of calcification, consisting of the deposit of fine

lime granules, in the middle part and more internal part of the media. This latter is not uniform and does not entirely enclose the vessel. Where the lime deposit is greatest, there is an abundant infiltration of small cells, some of which show polymorphic nuclei. The cells are in a poor state of preservation, and these focalized areas correspond more or less closely to the so-called atheromatous abscesses. A section stained for elastic tissue shows that the coarser elastic fibers remain but are especially prone to rupture in the course of section. In the location described as resembling atheromatous abscess, the clastic tissue is deficient or very much broken up. The smaller blood vessels about the aorta do not show any calcification.

The carotid artery shows a condition somewhat different to that of the aorta. The entire muscular coat of the artery has lost its nuclei. Not one remains. It is converted into an homogeneous structureless membrane richly infiltrated with lime salts. The adventitia is relatively free of calcification. The elastic tissue stain shows that the elastic framework remains but the fibrils have coalesced together into stands of greater or less thickness, showing in transverse sections a parallel arrangement. There is no fine network present.

No section has been made at the level of the most marked lesions which took place on the arch of the aorta, and on the pulmonary artery.

No attempt will be made to explain the genesis of these degenerative arterial changes. The problem contains too many unknown quantities, and hypothesis, however ingenious, would have very little objective value. Nevertheless a few details of this observation must be emphasized, especially as regards the time of occurrence of the calcification, its localization, and its relation to the renal lesions.

During the first fifteen days following the double transplantation the animal was in normal condition and no calcification took place as was shown by the anatomic conditions at the second laparotomy and by the normal structure of the scar of the first laparotomy. Then, the kidneys became suddenly enlarged, and the lesion noted at the laparotomy performed on the eighteenth day was œdema of all the transplanted tissues, for which no mechanical cause was discovered.

The calcification took place during the eighteen days which elapsed between the second laparotomy and the death of the animal.

The calcification was localized to the arteries, costal and bronchial cartilages, and to the points where the tissues had been disturbed by incision, or even merely by infiltration of a little blood. It was a remarkable fact that no calcification was found in any of the transplanted tissues. The transplanted segment of aorta,

and the renal arteries were normal. The subperitoneal connective tissue of the host and the connective tissue of the hilus of the right kidney were dissected during the second operation. However the connective tissue of the hilus was found normal at the autopsy, while the subperitoneal tissue was infiltrated with lime salts.

The exploratory incision of the right kidney was perfectly cicatrized, and there was no calcification at all of the renal tissue or the capsule. But at the level of the incision in the subperitoneal connective tissue there was a patch of lime salts. The blood, which flowed from the edges of the renal structures, had produced a deposit of lime salts in the tissue of the host, while it was harmless for the transplanted tissue.

It must be noticed also that the pathological changes undergone by the renal tissue were slight. However, the kidneys are evidently responsible for the occurrence of the arterial degeneration, directly or indirectly. Therefore, this observation seems to show that some unknown condition of the kidneys corresponding merely to unimportant morphological changes, can produce rapid, grave and extensive arterial lesions associated with deposits of lime salts in them and in other locations.